台灣農業研究

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol.74 No.3 2025

Feature Articles

205 Review of Black Soldier Fly (*Hermetia illucens* L.) as a Sustainable Protein Source in Aquaculture: Nutritional Limitations and Improvement Strategies
I-Pei Kuo, Yi-Jui Hsieh, and Shuenn-Der Yang

221 Egg Parasitoids as Important Natural Enemies of Auchenorrhynchan Insect Pests in East Asia
Serguei V. Triapitsyn

237 Breeding Methods for Improving the Resistance of *Apis mellifera* to *Varroa* Mite (*Varroa destructor*)
Pen-Han Chen, Zin-Hao Huang, Pei-Shou Hsu, Chi-Yen Pan, and Tzu-Hsien Wu

249 Temperature-Driven Dynamics of Development and Reproduction in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Insights for Advanced Pest Management
Jeong Joon Ahn, Jung-Eun Kim, Jong-Hwan Shin, and Yu-Bing Huang

265 Application of Hyperaccumulator Plants in the Remediation and Restoration of Heavy Metal Polluted Soils
Yi-Jing Chen and Chwen-Ming Yang

Research Articles

275 Survey of Passionfruit Viruses in Okinawa, Japan: Prevalence, Transmission, and Control of Passiflora Latent Virus Using Virus-Free Seedlings
Atsushi Ajitomi, Akari Saito, Shinji Kawano, and Ken-taro Sekine

291 Comparative Analysis of Xanthine Oxidase and Lipase Inhibition by Extracts from Different *Mesona chinensis* Cultivars
Tzu-Huan Hung, Chia-Hua Chang, Chiu-Hua Chen, Tsai-Li Kung, and Yang Yang

303 Effects of Long-Term Application of Pig Wastewater on Paddy Rice Production and Soil Properties
Hsi-Chia Wu, Chia-Jen Liu, Ya-Hui Shih, Min-Chieh Lin, Chung-Yi Liao, and Chi-Ling Chen

317 Preliminary Study on Multispectral Imaging Techniques for Early Pest Detection in Rice Production
Huai-Wen Tsao, Yu-Shun Huang, Shou-Horng Huang, Po-Yu Lai, and Ming-Hsin Lai

331 Integrated Disease Management to Reduce Disease Incidence in Exported Guava Fruits during Summer and Autumn
Shuen-Huang Tsai

345 Identification of *Phytophthora nicotianae*, the Causal Agent of Roselle Blight and Screening for Resistant Cultivars
Hui-Ling Tsai, Yue-Mei Zhan, Yi-Wei Hsu, Jin-Hsing Huang, Jyh-Nong Tsai, Pao-Jan Ann, Jing-Wen Chen, and Chu-Ping Lin

ISSN 2790-086X

Published by Taiwan Agricultural Research Institute, Ministry of Agriculture

台灣農業研究 JOURNAL OF TAIWAN AGRICULTURAL RESEARCH Vol.74 No.3 2025

ISSN 2790-086X
DOI:10.6156/JTAR

台灣農業研究

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol.74 No.3 2025

專題論述

205 黑水虻 (*Hermetia illucens*) 作為水產養殖永續性蛋白質原料的綜述：營養限制與改善策略
郭喬培、謝易叡、楊順德

221 頸喙亞目昆蟲的重要卵寄生蜂天敵
Serguei V. Triapitsyn

237 西方蜜蜂提高抗蜂蟹蟎能力之育種方法
陳本翰、黃子豪、徐培修、潘其彥、吳姿嫺

249 溫度驅動動態對東方果實蠅 (*Bactrocera dorsalis* (Hendel)) (雙翅目：果實蠅科) 發育與繁殖之探討：蟲害進階管理的見解
安晶駿、金廷恩、辛宗煥、黃毓斌

265 重金屬高富集植物在土壤重金屬污染整治與修復上之應用
陳怡菁、楊純明

研究報告

275 日本沖繩百香果病毒調查：百香果潛隱病毒的流行、傳播及利用去病毒苗達有效防治
Atsushi Ajitomi、Akari Saito、Shinji Kawano、Ken-taro Sekine

291 不同仙草品種萃取物對黃嘌呤氧化酶與脂肪酶抑制活性的比較分析
洪子桓、張家華、陳秋樺、龔財立、楊颺

303 長期施用養豬廢水對水稻生產與土壤性質之影響
吳錫家、劉嘉仁、施雅惠、林旻頡、廖崇億、陳琦玲

317 多光譜影像技術用於早期檢測水稻蟲害之研究
曹懷文、黃玉舜、黃守宏、賴柏羽、賴明信

331 降低外銷番石榴夏秋季果實罹病率之病害整合管理策略
蔡舜隍

345 洛神葵疫病病原 *Phytophthora nicotianae* 的鑑定及抗病品種之篩選
蔡惠玲、詹月梅、許珊瑋、黃晋興、蔡志濃、安寶貞、陳敬文、林筑蘋

農業部農業試驗所出版

《台灣農業研究》編審會

總編輯

石憲宗／農業試驗所應用動物組

副總編輯

吳東鴻／國立中興大學農藝學系
許健輝／農業試驗所農業化學組

領域主編 (按筆劃序)

王強生／國立中興大學農藝學系
古新梅／國立中興大學農藝學系
安寶貞／農業試驗所植物病理組
呂秀英／苗栗區農業改良場
杜宜殷／國立臺灣大學園藝暨景觀學系
林彥蓉／國立臺灣大學農藝學系
林慧玲／國立中興大學園藝學系
侯豐男／國立中興大學昆蟲學系
陳右人／國立臺灣大學園藝暨景觀學系
陳世銘／國立臺灣大學生物產業機電工程學系
陳健忠／農業試驗所應用動物組
陳琦玲／農業試驗所農業化學組
張永和／私立靜宜大學食品營養系
張瑞璋／農業藥物試驗所
莊愷瑋／國立中興大學農藝學系
郭寶錚／國立中興大學農藝學系
黃炳文／國立中興大學應用經濟學系
黃振文／國立中興大學植物病理學系
黃裕銘／國立中興大學土壤環境科學系
黃肇家／農業試驗所作物組
楊正澤／國立中興大學昆蟲學系
楊純明／農業試驗所作物組
路光暉／國立中興大學昆蟲學系
鄒裕民／國立中興大學土壤環境科學系
劉力瑜／國立臺灣大學農藝學系
蔡致榮／農業試驗所
鍾文鑫／國立中興大學植物病理學系
顏國欽／國立中興大學食品暨應用生物科技學系

英文主編 (按筆劃序)

王寅東／美國德州農工大學
張宗仁／美國喬治亞大學
楊景程／美國佛吉尼亞理工大學
鍾光仁／國立中興大學植物病理學系

邀請領域主編

鍾嘉綾／國立臺灣大學植物病理與微生物學系

編輯助理

王立蓉／《台灣農業研究》期刊編輯室

台灣農業研究

第七十四卷　第三期　　中華民國一一四年九月三十日

出版者　農業部農業試驗所
發行人　王仕賢
總編輯　石憲宗
地址　413008 臺中市霧峰區萬豐里中正路 189 號
網址　https://www.tari.gov.tw/jtar
電話　(04)2331-7236
E-mail　jtar@tari.gov.tw
線上投審稿系統　https://www.ipress.tw/J0042
台灣農業研究之稿約格式內容，詳自農業部農業試驗所首頁下列路徑下載：文宣出版 > 台灣農業研究 > 出版品稿約

編印　華藝學術出版部
地址　234634 新北市永和區成功路一段 80 號 18 樓
電話　(02)2926-6006
E-mail　press@airiti.com

定價　新臺幣一七五元
展售門市　1. 國家書店 / 104472 臺北市松江路 209 號 1 樓 / 電話 : (02)2518-0207
國家網路書店 http://www.govbooks.com.tw
2. 五南文化廣場 / 400002 臺中市中山路 6 號 / 電話 : (04)2226-0330

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):205–219 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0001

Feature Article

Review of Black Soldier Fly (*Hermetia illucens* L.) as a Sustainable Protein Source in Aquaculture: Nutritional Limitations and Improvement Strategies

I-Pei Kuo[1,*], Yi-Jui Hsieh[2], and Shuenn-Der Yang[3]

Abstract

Kuo, I. P., Y. J. Hsieh, and S. D. Yang. 2025. Review of black soldier fly (*Hermetia illucens* L.) as a sustainable protein source in aquaculture: Nutritional limitations and improvement strategies. J. Taiwan Agric. Res. 74(3):205–219.

Fishmeal is the primary protein source in aquaculture feed; however, due to limitations in marine resources, the global supply of fishmeal can no longer meet the rapidly growing demands of the aquaculture industry. Black soldier fly (*Hermetia illucens*) has the ability to efficiently convert inedible organic by-products into protein and is considered a sustainable alternative to fishmeal. However, black soldier fly larvae fed on plant-based substrates often face the nutritional limitations of low protein content and inadequate essential amino acids and fatty acids. These deficiencies may negatively impact fish growth performance, immune response, and flesh quality. Additionally, chitin, a major component of insect exoskeletons, has a controversial role in fish feed. While some studies indicate that chitin positively affects fish health, others have found that it acts as an anti-nutritional factor for certain fish species. This review evaluates the nutritional potential of black soldier fly meal in aquaculture feed. The primary limiting amino acids in black soldier fly meal include arginine, isoleucine, lysine, and methionine, while the major limiting fatty acids are eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). To enhance the nutritional quality of black soldier fly larvae, this review focuses on analyzing their protein content, amino acid and fatty acid profiles, and the effects of chitin. Furthermore, potential improvement strategies, including defatting processes, substrate manipulation, and chitin removal, are discussed.

Key words: Black soldier fly, Aquaculture, Amino acid, Fatty acid, Chitin.

INTRODUCTION

Aquaculture is a fast-growing industry that plays a key role in the food supply. In 2022, global aquaculture production reached 94 million tonnes, surpassing capture fisheries for the first time (FAO 2024). Fishmeal (FM), which contains 60–72% crude protein, has been used as a primary protein source in fish diets (Cho & Kim 2011; Luthada-Raswiswi *et al*. 2021). According to estimates from the Marine Ingredients Organisation, over 87% of FM was used in the aquaculture industry in 2021 (FAO 2024). However, the primary ingredients for FM production are wild-caught fish, such as anchovy (*Engraulis ringens* J.), Jack mackerel (*Trachurus murphyi* N.), sardine (*Strangomera bentincki*), menhaden (*Brevoortia tyrannus*), and Alaska pollock (*Theragra chalcogramma*) (Hall 2010). Due to overfishing, climate

Received: January 20, 2025; Accepted: April 6, 2025.
* Corresponding author, e-mail: ipkuo@mail.ntou.edu.tw
1 Assistant Professor, Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan, ROC.
2 Associate Research Fellow, Penghu Fisheries Biology Research Center, Fisheries Research Institute, Penghu County, Taiwan, ROC.
3 Research Fellow, Freshwater Aquaculture Research Center, Fisheries Research Institute, Changhua County, Taiwan, ROC.

change, and ocean pollution, the limited supply of FM is insufficient to meet aquaculture demands (Shahidul Islam & Tanaka 2004; Olsen & Hasan 2012; Jannathulla *et al*. 2019). Therefore, developing alternatives to FM is crucial for ensuring sustainability in aquaculture.

Regarding protein content, environmental sustainability, consumer acceptance, and feasibility, insect meal is one of the most promising alternatives to FM (Hua *et al*. 2019). Among various insects, black soldier fly (BSF; *Hermetia illucens*) has received significant research attention due to its outstanding efficiency and biosecurity. BSF can convert organic waste into insect protein with a superior feed conversion ratio (1.4–2.6), compared to other insects, such as yellow mealworm (*Tenebrio molitor*; 3.8–19.1) and house cricket (*Acheta domesticus*; 2.3–6.1) (Oonincx *et al*. 2015). Secondly, no entomopathogen outbreak has been documented in BSF production up to the present. Their high resistance to disease infection reduces the risk in commercial production (Joosten *et al*. 2020). Thirdly, a feeding trial revealed that the BSF does not exhibit bioaccumulation effects for mycotoxin (Deoxynivalenol, Aflatoxin B1/B2/G2, Ochratoxin A, and Zearalenone) and pesticide (Chlorpyrifos, Chlorpyrifos-methyl, Pirimiphos-methyl). Moreover, the reduced pesticide levels detected in the residual substrate suggested that BSF may have the ability to degrade pesticides (Purschke *et al*. 2017). Further studies have proved that the ability of BSF to degrade Aflatoxin B1 and antibiotics (ciprofloxacin, tylosin, and enrofloxacin) primarily stems from the microorganisms in its gut (Mei *et al*. 2022; Yang *et al*. 2022; Suo *et al*. 2023).

BSF belongs to the phylum Arthropoda, class Insecta, order Diptera, and family Stratiomyidae. The life cycle of BSF is divided into five stages, namely egg, larvae (five instars), prepupae (sixth instar), pupae, and adult. The adults-only consume water and do not have stingers. Therefore, they are regarded as harmless insects to humans and can even be reared in residential areas for organic waste disposal. The larva is the only stage that consumes food and is generally reared for 2–4 wk (Basri *et al*. 2022; Purkayastha & Sarkar 2022). Upon developing into the prepupae stage, they stop feeding and migrate from the substrate. Leveraging this behavior, the technology of self-harvesting can be developed to reduce operational costs (Giannetti *et al*. 2022).

Research on replacing FM with BSF meal has been extensively conducted. However, the optimal FM replacement level varies greatly across different studies. The potential adverse effects of excessive dietary inclusion of BSF on fish include growth retardation, intestinal damage, coloration reduction, nutrition composition decline, and flesh quality impairment (Huang *et al*. 2022; Khieokhajonkhet *et al*. 2022; Kuo *et al*. 2022; Yamamoto *et al*. 2022). In addition to differences between fish species, the nutritional requirements of fish are also determined by various factors, such as growth stage, water quality, feeding regime, and feed processing condition (Jobling 2016). However, the lack of standardization in BSF meal quality may be a greater challenge for feed manufacturing due to their high plasticity in nutritional profile (Barragan-Fonseca *et al*. 2021; Siva Raman *et al*. 2022). Several studies have shown that the proximate composition, fatty acid profile, and amino acid profile can be manipulated by feeding substrates (Liland *et al*. 2017; Fuso *et al*. 2021; Rodrigues *et al*. 2022). In addition, the harvest stage and processing method are also crucial factors that affect the quality of BSF meal (Huang *et al*. 2019; Do *et al*. 2020).

Although BSF meal is regarded as a potential FM alternative and has been commercially produced, its grading standard has not yet been established. Since only a few studies address the quality of BSF from the perspective of aquaculture, this literature review aims to provide an overview of its limited nutritional value and summarize a possible improvement strategy based on fish’s nutritional requirements.

NUTRITIONAL VALUE

Proximate composition

The proximate composition and chitin content of BSF fed on various substrates are listed in Table 1. The body composition of BSF is greatly altered by the feeding substrates. The crude protein (conversion factor of 6.25) and lipid content of BSF ranged from 30.8–59.8% and 21.6–40.7%, respectively. The highest protein content was observed in the BSF fed on brewery by-products. Moreover, a correlation matrix between the proximate composition of substrates and the body composition of BSF is illustrated in Fig. 1. The crude protein content of substrates was positively correlated with the crude protein of BSF ($P < 0.05$). However, a negative correlation was found between the crude protein of substrates and the crude lipid of BSF ($P < 0.05$). These results indicate that feeding BSF with low-protein substrates leads to lower protein but higher lipid accumulation.

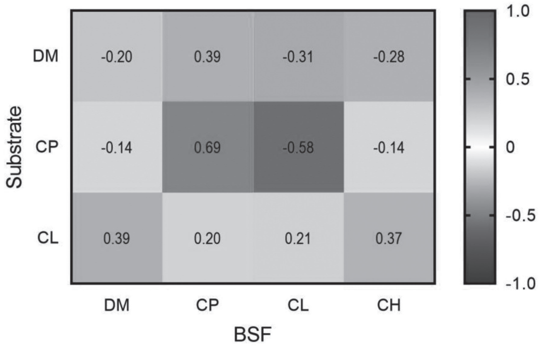

Fig. 1. Correlation matrix between the proximate composition of substrates and the body composition of black soldier fly (BSF). DM: dry matter; CP: crude protein; CL: crude lipid; CH: chitin. Values in the cells represents the Pearson's correlation coefficients.

Table 1. Proximate composition and chitin content of black soldier fly (BSF) fed with various substrates.

	Substrate[z]			BSF				
Feeding substrates	DM	CP	CL	DM	CP	CL	Chitin	Reference
Food waste	17.7	20.2	19.4	41.0	39.5	38.1	8.1	Hosseindoust *et al*. (2023)
Tofu byproduct	24.1	29.2	11.2	42.9	43.5	37.0	8.7	
Vegetable	11.1	13.1	8.6	38.0	37.7	34.5	7.1	
Chicken feed	25.8	17.5	5.3	38.7	41.2	33.6	6.2	Spranghers *et al*. (2017)
Digestate	24.3	24.6	6.2	38.6	42.2	21.8	5.6	
Vegetable waste	12.7	8.6	2.1	41.0	39.9	37.1	5.7	
Restaurant waste	26.2	15.7	13.9	38.1	43.1	38.6	6.7	
Vegetable + fruit waste[y]	8.3	12.0	2.6	22.0	41.9	26.3	6.2	Meneguz *et al*. (2018)
Fruit waste	13.2	4.6	2.8	28.3	30.8	40.7	5.6	
Winery byproduct	35.8	11.7	7.9	26.5	34.4	32.2	5.3	
Brewery byproduct	23.2	20.1	8.7	29.1	53.0	29.9	1.4	
Chicken feed	31.2	19.5	4.5	29.5	43.6	32.9	5.1	Eggink *et al*. (2022a)
Mixed feed[x]	28.7	20.6	3.4	26.2	43.4	27.0	5.1	
Brewery by-product	31.2	26.1	9.8	22.8	59.8	22.4	4.7	
Mussels	26.2	19.9	3.4	20.6	48.4	21.6	2.6	
Rapeseed cake	31.7	30.9	12.7	29.1	51.5	24.5	4.7	
Shrimp waste	20.0	37.6	7.4	21.8	49.5	22.9	4.1	

[z] DM: dry matter; CP: crude protein; CL: crude lipid. The proximate composition and chitin content were presented as the percentage of DM. A nitrogen-to-protein conversion factor of 6.25 was used for CP calculation.

[y] 70% vegetable and 30% fruit waste.

[x] Mixed feed was formulated with 66.5% water, 16.0% pea grits, 8.0% wheat, 7.0% chicken starter feed, 2.1% sugar beet pellet, and 0.4% vitamin-mineral mixture.

Due to its high content of unsaturated fatty acids, BSF fat is susceptible to oxidation during storage or feed processing, leading to reduced palatability and adverse physiological effects (Jorge Iñaki *et al*. 2022).

Protein is the most expensive nutrient and accounts for 30–50% of fish diet (Aragão *et al*. 2022). Compared to terrestrially-derived proteins, marine-based proteins offer a more balanced amino acid profile for fish but are more costly (Ma *et al*. 2020). High-quality FM generally contains 60–72% crude protein but remains only 6–10% of crude lipid (Cho & Kim 2011). To replace FM in fish diet, insufficient protein levels and deficiency in essential amino acids are the primary limitations for BSF protein. In commercial feed production, oil is generally coated after pelleting to prevent oxidation from high temperatures during extrusion (Chaabani *et al*. 2020). Therefore, the development of defatting and amino acid manipulation technologies is recommended to enhance the quality of BSF protein.

Amino acids

The essential amino acids in fish, namely arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine, are pivotal for growth and metabolism (Xing *et al*. 2024). The essential amino acid profile of various insect meals is presented in Table 2. Compared with FM, the essential amino acid index (EAAI) of BSF, cricket, and mealworm are 1.01, 0.89, and 0.58, respectively, indicating that BSF has a relatively balanced amino acid profile than others. The chemical score of BSF shows that arginine is the first limiting amino acid, followed by isoleucine, lysine, and methionine.

Soybean meal is a conventional plant-based protein source and has been successfully used as an FM alternative in various fish diets (Zhou *et al*. 2005; Lim *et al*. 2011; Yang *et al*. 2011). However, the primary limiting amino acid of soybean meal is methionine, with a chemical score of only 0.51. Methionine is also insufficient in BSF. Additionally, using crystalline amino acids is low in fish (Dabrowski *et al*. 2010). Therefore, the methionine content in BSF is a critical factor in determining its suitability for replacing FM.

Cystine is not an essential amino acid as it can be synthesized from methionine. Therefore, a sufficient dietary intake of cysteine can spare methionine (Twibell *et al*. 2000). Recent study demonstrated that there was a positive linear regression between methionine content in BSF larvae and rearing time, while no linear regression was observed in BSF larvae cysteine concerning rearing time. Moreover, elevating the carbon-to-nitrogen (C/N) ratio in almond hulls also facilitated methionine accumulation in BSF larvae but did not affect their cysteine content (Miner *et al*. 2022). This result indicates that the methionine content in BSF larvae can be manipulated by feeding substrates. However, the unchanged cysteine content suggests that the biosynthesis pathway of BSF may differ from that of vertebrates.

Fatty acid

Lipids are the most energy-dense macronutrient (9 kcal g^{-1}) and can effectively reduce protein loss by catabolism (Thirunavukkarasar *et al*. 2022). As shown in Table 3, the predominant fatty acid in BSF is lauric acid (C_{12} : 0). When the rapeseed oil was replaced with BSF oil in the Atlantic salmon (*Salmo salar*) diet, the apparent digestibility coefficients of lauric acid reached 95% and higher. In comparison, approximately only 50% of ingested lauric acid was recuperated in the tissues, indicating lauric acid is highly oxidized for energy generation. In addition, the high level of lauric acid in the BSF-based diets prevented fish liver from excessive lipid storage, which reflects metabolism dysfunction (Belghit *et al*. 2019b). These results showed that lauric acid derived from BSF is a potential protein sparer.

Furthermore, lauric acid possesses a broadly antimicrobial ability against various fish pathogens and can alleviate the inflammation induced by a bacterial infection (Huang *et al*. 2014; do Couto *et al*. 2021). Supplementing diets

Table 2. Amino acid profile of fishmeal (FM), soybean meal, and various insect meals.

Amino acid profile	FM[z]	Soybean Meal[y]	BSF[x]	Cricket[w]	Mealworm[v]
g 16 g^{-1} nitrogen					
Arginine	7.86	7.23	4.93	6.20	3.61
Histidine	1.84	2.53	3.30	2.20	1.60
Isoleucine	6.12	4.54	4.17	3.75	2.51
Leucine	6.85	7.78	6.94	6.70	4.22
Lysine	8.18	6.38	5.68	5.14	3.03
Methionine	2.49	1.26	1.84	2.02	1.15
Phenylalanine	3.55	4.94	4.13	4.00	2.51
Threonine	4.00	3.86	3.98	3.88	2.42
Tryptophan	0.72	1.28	1.63	0.85	0.57
Valine	5.33	4.80	5.85	4.24	3.62
Chemical score[u]					
Arginine	-	0.92	0.63	0.79	0.46
Histidine	-	1.38	1.79	1.20	0.87
Isoleucine	-	0.74	0.68	0.61	0.41
Leucine	-	1.14	1.01	0.98	0.62
Lysine	-	0.78	0.69	0.63	0.37
Methionine	-	0.51	0.74	0.81	0.46
Phenylalanine	-	1.39	1.16	1.13	0.71
Threonine	-	0.97	1.00	0.97	0.61
Tryptophan	-	1.78	2.26	1.18	0.79
Valine	-	0.90	1.10	0.80	0.68
EAAI[u]	-	0.99	1.01	0.89	0.58

[z] Whole herring meal (Boge 1960). FM was used as the reference protein.
[y] Egyptian soybean (Saleh 2020).
[x] Black soldier fly (BSF) prepupae fed with chicken feed (Spranghers *et al*. 2017).
[w] *Gryllus bimaculatus* (Taufek *et al*. 2018).
[v] *Tenebrio molitor* (Wu *et al*. 2020).
[u] Chemical score and essential amino acid index (EAAI) were calculated according to Kirimi *et al*. (2020).

with lauric acid (0.1% and 0.8%) in black sea bream (*Acanthopagrus schlegelii*) improved the growth performance, antioxidative capability, and intestinal morphology and microbiota of fish (Ullah *et al*. 2022). Similarly, replacing 25% of soybean oil with BSF oil facilitated the growth performance of Nile tilapia (*Oreochromis niloticus*) fry without compromising the fish body's fatty acid profile or hematological homeostasis (Goda *et al*. 2024).

However, arachidonic acid (ARA, C_{20} : 4n - 6), eicosapentaenoic acid (EPA, C_{20} : 5n - 3), and docosahexaenoic acid (DHA, C_{22} : 6n - 3), which are essential fatty acids for fish, are deficient in BSF. Since ARA is abundant in poultry meat (2.4–10.4%) (Kawashima 2019), the deficiency of ARA in BSF can be addressed through supplementation with poultry meal. By contrast, EPA and DHA are mainly originated from the aquatic ecosystems (Gladyshev *et al*. 2013). These fatty acids are generally deficient in land plants and poultry. Consequently, EPA and DHA are the primary limiting factors in meeting the essential fatty acid requirements of fish. Several studies have validated that replacing FM with BSF meal leads to poor EPA

Table 3. Fatty acid profile of cod liver oil, soybean oil, and black soldier fly (BSF).

% of total identified fatty acid	Cod liver oil[z]	Soybean oil[y]	BSF[x]
10 : 0	N.D.[w]	N.D.	1.58
12 : 0	N.D.	N.D.	46.39
14 : 0	6.38	0.08	7.85
14 : 1n - 5	N.D.	N.D.	0.53
16 : 0	19.09	11.49	12.21
16 : 1n - 7	8.75	N.D.	5.56
18 : 0	4.76	4.11	1.61
18 : 1n - 9	21.09	23.73	10.59
18 : 2n - 6	4.74	55.61	12.46
18 : 3n - 3	0.98	4.97	0.99
20 : 1n - 9	2.16	N.D.	N.D.
20 : 2n - 6	0.35	N.D.	N.D.
20 : 3n - 6	0.21	N.D.	N.D.
20 : 4n - 6	0.85	N.D.	N.D.
20 : 4n - 3	1.41	N.D.	N.D.
20 : 5n - 3	9.68	N.D.	0.23
21 : 0	1.11	N.D.	N.D.
22 : 5n - 3	4.45	N.D.	N.D.
22 : 6n - 3	14.00	N.D.	N.D.
SFA[w]	31.34	15.68	69.64
MUFA[w]	32.00	23.73	16.68
PUFA[w]	36.67	60.59	13.68
n - 3	30.52	4.97	1.22
n - 6	6.14	55.61	12.46
n - 3/n - 6	4.97	0.09	0.10

[z] Data from Francis *et al*. (2006).
[y] Data from Ferreira *et al*. (2016).
[x] BSF prepupae fed with wheat bran (El-Dakar *et al*. 2020).
[w] N.D.: not detected; SFA: saturated fatty acids; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acids.

and DHA accumulation in fish bodies (Mancini *et al*. 2018; Guerreiro *et al*. 2020; Oteri *et al*. 2022; Takakuwa *et al*. 2022). Clinical signs of essential fatty acid deficiency in fish include poor growth rate, increased mortality, fin erosion, myocarditis, and sensitivity to stress (Glencross 2009). Besides animal welfare, essential fatty acids are crucial to product quality. EPA and DHA are common indicators to assess the nutritional value of seafood (Chen & Liu 2020). Moreover, a recent study found that salmon fed low levels of EPA and DHA had higher metabolic rates of astaxanthin and poor fillet color (Ytrestøyl *et al*. 2023).

Overall, BSF contains a predominant amount of lauric acid with various biofunctions but lacks EPA and DHA. Therefore, feed formulations should be designed based on the nutritional requirements of fish. In general, marine fish lack the ability to convert short-chain polyunsaturated fatty acids (PUFA) to long-chain PUFA; by contrast, freshwater fish can utilize n - 3 and n - 6 C-18 PUFA to synthesize more highly unsaturated long-chain PUFA. Consequently, marine fish have a higher requirement for n - 3 long-chain PUFAs than freshwater fish (Glencross 2009). For instance, dietary BSF-based diet resulted in the lower accumulation of n - 3 long-chain PUFAs in various marine fish, such as meagre (*Argyrosomus regius*), gilthead sea bream (*Sparus aurata*), and red sea bream (*Pagrus major*) (Guerreiro *et al*. 2020; Oteri *et al*. 2022; Takakuwa *et al*. 2022). Comparatively, completely replacing fish oil (FO) with BSF oil did not compromise the DHA content in the flesh of red hybrid tilapia (*Oreochromis* sp.) (Abu Bakar *et al*. 2021).

Chitin

Chitin is a linear polymer consisting of N-acetylglucosamine units linked by β-1,4 glycosidic bonds and presents in the exoskeleton of insects (Shamshina *et al*. 2019). As shown in Table 1, the chitin content is in the range of 1.4–8.7%. The Kjeldahl method is a widely used technique for determining the crude protein content. It estimates nitrogen content, which is then converted to crude protein content using a conversion factor. Because chitin is a nitrogen-containing compound, the crude protein of BSF meal may be overestimated when a general factor of 6.25 is used. Considering nitrogen from the chitin and other non-protein compound, conversion factors are corrected to 4.76, 4.21–5.01, and 4.47 by Janssen *et al*. (2017), Belghit *et al*. (2019a) and Smets *et al*. (2021), respectively.

Although most fish have been shown to possess chitin-degrading enzymes, their utili-

zation of chitin varies among species. Dietary 2.5–10.0% of the chitin extracted from crab shells promoted the non-specific immunity of gilthead seabream (*Sparus aurata*), including natural haemolytic complement activity, respiratory burst activity, and natural cytotoxic activity, without any negative effect on the growth performance (Esteban *et al*. 2001). By contrast, both chitin and chitosan retarded the growth performance of hybrid tilapia (*O. niloticus* × *O. aureus*) at the inclusion levels between 2–10% (Shiau & Yu 1999). In rainbow trout (*Oncorhynchus mykiss*), the tolerance of chitin derived from shrimp shells was 3%. Excessive chitin inclusion led to growth inhibition and reduced nutrient digestibility (Pascon *et al*. 2024).

Short-chain fatty acids (SCFAs), such as acetate, propionate, and butyrate, are the predominant products of bacterial chitin degradation and contribute to fish intestinal health. Therefore, chitin can potentially serve as a prebiotic in fish diets (Hasan *et al*. 2023). However, the source of chitin is a crucial factor in bioavailability. Recent studies have revealed that insects are a superior chitin source compared to crustaceans. Dietary pupal BSF exuviae meal had greater effects on intestinal SCFA-producing bacteria and SCFA production compared to shrimp head meal in rainbow trout (Rimoldi *et al*. 2023). Similarly, in largemouth bass (*Micropterus salmoides*), dietary chitin derived from both BSF and shrimp exerted positive effects on antioxidant status, non-specific immunity, intestinal microbiota, and SCFA production; however, only BSF chitin further improved growth performance and monounsaturated fatty acid deposition in muscle tissue (Hu *et al*. 2025).

Despite its prebiotic effects, chitin has also been reported to exhibit anti-nutritional properties in fish diets (Eggink *et al*. 2022b). An *in vitro* two-step enzymatic assay using pepsin and trypsin-enriched pancreatin revealed that the crude protein digestibility of BSF meal was negatively correlated with the chitin content, which was the primary variable identified in the stepwise analysis (Marono *et al*. 2015). Therefore, further studies to investigate the effect of BSF chitin on different fish species and to establish their tolerance dietary level are recommended.

IMPROVEMENT STRATEGY

Defatting

The high lipid content and abundance of unsaturated fatty acids in whole insect meal make it prone to oxidation, which reduces its palatability. Therefore, defatting is an effective process to stabilize the quality of BSF meal (Henry *et al*. 2015). When the BSF meal was used as an FM alternative in the gilthead seabream diet, the defatted meal was more readily accepted by fish than the full-fat meal (Karapanagiotidis *et al*. 2023). The defatting process is also beneficial to fish growth. Replacing FM at an isoproteic level with an adjusted fatty acid profile, the removal of the fat fraction from BSF meal improved the growth of yellowtail (*Seriola quinqueradiata*), indicating that factors in BSF oil other than fatty acids affect growth (Ido *et al*. 2021). Similarly, when 14% of FM protein was replaced in a turbot (*Scophthalmus maximus*) diet, full-fat BSF meal retarded growth performance and altered intestinal microbiota composition by increasing the abundance of potential pathogenic bacteria, whereas defatted BSF meal did not compromise these aspects and showed similarity to the FM-based diet (Zhao *et al*. 2023).

The conventional defatting processes of insect meal are divided into chemical and mechanical methods. Chemical defatting uses organic solvents, such as ethanol, acetone, and hexane, to remove insect oil. Although chemical defatting can achieve oil recovery, the residual organic solvents result in environmental or hygienic concerns (Kim *et al*. 2022). Mechanical defatting extracts oil using screw and hydraulic press in small-scale and mass production, respectively. Without pre-heating,

cold-pressing directly mechanical presses the ingredient where the temperature only rises to 50–60℃ for a short time (Leming & Lember 2005).

Comparing the quality of BSF meal defatted using chemical (aqueous, acetone, ethanol, and hexane) extraction and mechanical (cold-pressing) defatting methods, the cold-pressing showed higher total essential amino acid content and emulsifying capacity than chemical extraction (Kim *et al*. 2022). Likewise, BSF meal defatted with the mechanical method had higher digestibility of lipids, leucine, and arginine than a chemical method in hens (Xin *et al*. 2024). In terms of BSF oil, pressing and supercritical carbon dioxide extraction led to lower peroxide value than hexane after extraction (Hurtado-Ribeira *et al*. 2023). Overall, these studies indicated that the mechanical method is superior to the chemical to produce defatted BSF meal and BSF oil.

Enrichment

Rearing BSF with single substrates generally results in nutrient deficiency (Raksasat *et al*. 2020). To improve nutrient deficiency, several enrichment approaches have been studied recently. Substituting 40% of commercial laying hen feed with FM resulted in the EPA and DHA content of BSF larvae reaching a maximum level of 0.8% and 1.9%, respectively, after 3 h of enrichment. Moreover, the peak in their crude protein content (increased from 36.7 to 55.2%) was observed after 24 h (Barroso *et al*. 2017). BSF larvae reared on a fish-based substrate (soybean meal and marine fish offal) were enriched in EPA and DHA (2.2% and 0.4%, respectively), resulting in better growth in Nile tilapia compared to unenriched BSF meal when used as a replacement for FM and FO (Agbohessou *et al*. 2024). A similar result was also reported in rainbow trout, in which fish offal-enriched BSF meal, but not unenriched BSF meal could replace FM up to 50% without causing growth inhibition (Sealey *et al*. 2011). In addition, the inclusion of 25% fish waste in banana and orange peels resulted in a 2.7-fold increase in biomass conversion efficiency, indicating that co-composting plant and fish byproducts can not only improve nutrient availability but also reduce the production cost of BSF meal (Isibika *et al*. 2021).

Algae are the predominant producers of EPA and DHA in aquatic ecosystems. The brown seaweed (*Ascophyllum nodosum*) was used to replace the basic feeding medium at levels ranging 0% to 100%. The EPA content of BSF larvae was positively correlated with the dietary level of brown seaweed. However, it is worth noting that dietary brown seaweed negatively affected the growth performance of BSF (Liland *et al*. 2017). Furthermore, since seaweed can accumulate heavy metals from the environment, the cadmium and total arsenic levels in BSF larvae exceeded the maximum residue limits set by the European Union when the dietary level of brown seaweed exceeded 20% (Biancarosa *et al*. 2018).

Rearing BSF larvae on chicken manure, brewers' spent grain, and kitchen waste modified their arginine, glutamic acid, proline, tyrosine, and phenylalanine, whereas no effect was detected on the most limiting amino acids, such as lysine, methionine, isoleucine, and tyrosine (Shumo *et al*. 2019). Another study showed that lysine, valine, and leucine were the most altered amino acids in BSF larvae when fed different vegetable by-products (Fuso *et al*. 2021). A single amino acids reduction trial demonstrated that reducing 65% of each amino acid did not affect the growth performance of BSF larvae. Moreover, balance calculations for individual amino acids revealed that deficiency of amino acids was compensated through substrate microbiome synthesis (Lemme & Klüber 2024).

As discussed in Section Amino acid, methionine content in BSF is a critical limiting amino acid for FM replacement. However, to date, no study has demonstrated that BSF larvae are capable of directly accumulating methionine from methionine-enriched substrates. Extending rearing time and increasing

the C/N ratio in substrates have been proven to enhance methionine levels in BSF larvae, indicating that methionine content can be improved through methods other than direct enrichment (Miner *et al*. 2022). Moreover, since microbiota play a crucial role in amino acid synthesis in BSF substrates (Lemme & Klüber 2024), further studies are needed to identify the amino acid-synthesizing bacteria.

Removal of chitin

The fractionation of BSF typically includes dry and wet modes. The dry mode is a conventional processing method of insects, which involves drying and defatting steps. The dry mode cannot isolate chitin from the insect body and the final products are BSF oil and defatted BSF meal. By contrast, the wet mode uses a juice press step to separate the chitin-rich press cake from BSF juice. Subsequently, the BSF juice is centrifuged and divided into the lipid, cream, aqueous, and solid fractions. The composition analysis revealed that approximately 60% of the total chitin is retained in the press cake, and the remaining chitin (40%) is primarily found in the solid fraction (Ravi *et al*. 2021).

Sieving is an alternative method for fractionation. The BSF meal defatted using the pressing method was mechanically sieved into particle sizes of 0–200 µm, 200–400 µm, and > 400 µm. Their chitin contents were 18, 27, and 154 g kg^{-1}, respectively, with similar crude protein levels of 54.9%, 55.7%, and 55.9%, respectively. This result indicated that a 400 µm sieve can effectively filter out the chitin-rich fraction. However, the yield of each fraction was not provided in that study (Eggink *et al*. 2022b). To assess the feasibility of sieving for fractionation, further studies on yield and optimal particle sizes are necessary.

CONCLUSION

BSF has emerged as a promising and sustainable alternative protein source to FM in aquaculture. However, some nutritional limitations, particularly in essential amino acids (e.g., methionine), essential fatty acids (e.g., EPA and DHA), and excessive chitin must be addressed to optimize its use in aquafeeds. Strategies such as defatting, substrate enrichment, and chitin removal can enhance the nutritional quality and bioavailability of BSF meals to fish. To maximize its potential as a sustainable feed ingredient in the aquaculture industry, establishing BSF meal quality standards and conducting additional studies on species-specific dietary tolerance are necessary.

ACKNOWLEDGMENTS

This review was supported by the Fisheries Research Institute of Taiwan (114AS-6.1.5-AI-01).

REFERENCES

Abu Bakar, N. H., S. Abdul Razak, N. Mohd Taufek, and Z. Alias. 2021. Evaluation of black soldier fly (*Hermetia illucens*) prepupae oil as meal supplementation in diets for red hybrid tilapia (*Oreochromis* sp.). Intl. J. Trop. Insect Sci. 41:2093–2102. doi:10.1007/s42690-020-00398-z

Agbohessou, P. S., R. Mandiki, W. Mes, A. Blanquer, M. Gérardy, M. M. Garigliany, ... P. Kestemont. 2024. Effect of fatty acid-enriched black soldier fly larvae meal combined with chitinase on the metabolic processes of Nile tilapia. Br. J. Nutr. 131:1326–1341. doi:10.1017/S0007114523003008

Aragão, C., A. T. Gonçalves, B. Costas, R. Azeredo, M. J. Xavier, and S. Engrola. 2022. Alternative proteins for fish diets: Implications beyond growth. Animals 12:1211. doi:10.3390/ani12091211

Barragan-Fonseca, K. B., G. Gort, M. Dicke, and J. J. A. van Loon. 2021. Nutritional plasticity of the black soldier fly (*Hermetia illucens*) in response to artificial diets varying in protein and carbohydrate concentrations. J. Insects Food Feed 7:51–61. doi:10.3920/JIFF2020.0034

Barroso, F. G., M. J. Sánchez-Muros, M. Segura, E. Morote, A. Torres, R. Ramos, and J. L. Guil. 2017. Insects as food: Enrichment of larvae of *Hermetia illucens* with omega 3 fatty acids by means of dietary modifications. J. Food Compos. Anal. 62:8–13. doi:10.1016/j.jfca.2017.04.008

Basri, N. E. A., N. A. Azman, I. K. Ahmad, F. Suja, N. A. A. Jalil, and N. F. Amrul. 2022. Potential applications of frass derived from black soldier fly larvae treatment of food waste: A review. Foods 11:2664. doi:10.3390/foods11172664

Belghit, I., E. J. Lock, O. Fumière, M. C. Lecrenier, P. Renard, M. Dieu, ... J. D. Rasinger. 2019a. Species-specific discrimination of insect meals for aquafeeds by direct comparison of tandem mass spectra. Animals 9:222. doi:10.3390/ani9050222

Belghit, I., R. Waagbø, E. J. Lock, and N. S. Liland. 2019b. Insect-based diets high in lauric acid reduce liver lipids in freshwater Atlantic salmon. Aquac. Nutr. 25:343–357. doi:10.1111/anu.12860

Biancarosa, I., N. S. Liland, D. Biemans, P. Araujo, C. G. Bruckner, R. Waagbø, ... H. Amlund. 2018. Uptake of heavy metals and arsenic in black soldier fly (*Hermetia illucens*) larvae grown on seaweed-enriched media. J. Sci. Food Agric. 98:2176–2183. doi:10.1002/jsfa.8702

Boge, G. 1960. Amino-acid composition of herring (*Clupea harengus*) and herring meal. Destruction of amino-acids during processing. J. Sci. Food Agric. 11:362–365. doi:10.1002/jsfa.2740110703

Chaabani, A., L. Labonne, C. A. Tercero, J. P. Picard, C. Advenier, V. Durrieu, ... P. Evon. 2020. Optimization of vacuum coating conditions to improve oil retention in trout feed. Aquac. Eng. 91:102127. doi:10.1016/j.aquaeng.2020.102127

Chen, J. and H. Liu. 2020. Nutritional indices for assessing fatty acids: A mini-review. Intl. J. Mol. Sci. 21:5695. doi:10.3390/ijms21165695

Cho, J. H. and I. H. Kim. 2011. Fish meal- Nutritive value. J. Anim. Physiol. Anim. Nutr. 95:685–692. doi:10.1111/j.1439-0396.2010.01109.x

Dabrowski, K., Y. F. Zhang, K. Kwasek, P. Hliwa, and T. Ostaszewska. 2010. Effects of protein-, peptide- and free amino acid-based diets in fish nutrition. Aquac. Res. 41:668–683. doi:10.1111/j.1365-2109.2010.02490.x

Do, S., L. Koutsos, P. L. Utterback, C. M. Parsons, M. R. C. de Godoy, and K. S. Swanson. 2020. Nutrient and AA digestibility of black soldier fly larvae differing in age using the precision-fed cecectomized rooster assay. J. Anim. Sci. 98:skz363. doi:10.1093/jas/skz363

do Couto, M. V. S., N. da Costa Sousa, P. E. G. Paixão, E. dos Santos Medeiros, H. A. Abe, J. O. Meneses, ... R. Y. Fujimoto. 2021. Is there antimicrobial property of coconut oil and lauric acid against fish pathogen? Aquaculture 545:737234. doi:10.1016/j.aquaculture.2021.737234

Eggink, K. M., I. Lund, P. B. Pedersen, B. W. Hansen, and J. Dalsgaard. 2022a. Biowaste and by-products as rearing substrates for black soldier fly (*Hermetia illucens*) larvae: Effects on larval body composition and performance. PLOS ONE 17:e0275213. doi:10.1371/journal.pone.0275213

Eggink, K. M., P. B. Pedersen, I. Lund, and J. Dalsgaard. 2022b. Chitin digestibility and intestinal exochitinase activity in Nile tilapia and rainbow trout fed different black soldier fly larvae meal size fractions. Aquac. Res. 53:5536–5546. doi:10.1111/are.16035

El-Dakar, M. A., R. R. Ramzy, H. Ji, and M. Plath. 2020. Bioaccumulation of residual omega-3 fatty acids from industrial *Schizochytrium* microalgal waste using black soldier fly (*Hermetia illucens*) larvae. J. Clean. Prod. 268:122288. doi:10.1016/j.jclepro.2020.122288

Esteban, M. A., A. Cuesta, J. Ortuño, and J. Meseguer. 2001. Immunomodulatory effects of dietary intake of chitin on gilthead seabream (*Sparus aurata* L.) innate immune system. Fish Shellfish Immunol. 11:303–315. doi:10.1006/fsim.2000.0315

Ferreira, E. M., A. V. Pires, I. Susin, M. V. Biehl, R. S. Gentil, M. O. M. Parente, ... E. de Almeida. 2016. Nutrient digestibility and ruminal fatty acid metabolism in lambs supplemented with soybean oil partially replaced by fish oil blend. Anim. Feed Sci. Technol. 216:30–39. doi:10.1016/j.anifeedsci.2015.09.007

Food and Agriculture Organization of the United Nations (FAO). 2024. The State of World Fisheries and Aquaculture 2024 – Blue Transformation in Action. FAO. Rome, Italy. 264 pp. doi:10.4060/cd0683en

Francis, D. S., G. M. Turchini, P. L. Jones, and S. S. De Silva. 2006. Effects of dietary oil source on growth and fillet fatty acid composition of Murray cod, *Maccullochella peelii peelii*. Aquaculture 253:547–556. doi:10.1016/j.aquaculture.2005.08.008

Fuso, A., S. Barbi, L. I. Macavei, A. V. Luparelli, L. Maistrello, M. Montorsi, ... A. Caligiani. 2021. Effect of the rearing substrate on total protein and amino acid composition in black soldier fly. Foods 10:1773. doi:10.3390/foods10081773

Giannetti, D., E. Schifani, R. Reggiani, E. Mazzoni, M. C. Reguzzi, C. Castracani, ... D. A. Grasso. 2022. Do it by yourself: Larval locomotion in the black soldier fly *Hermetia illucens*, with a novel "self-harvesting" method to separate prepupae. Insects 13:127. doi:10.3390/insects13020127

Gladyshev, M. I., N. N. Sushchik, and O. N. Makhutova. 2013. Production of EPA and DHA in aquatic ecosystems and their transfer to the land. Prostaglandins Other Lipid Mediat. 107:117–126. doi:10.1016/

j.prostaglandins.2013.03.002

Glencross, B. D. 2009. Exploring the nutritional demand for essential fatty acids by aquaculture species. Rev. Aquac. 1:71–124. doi:10.1111/j.1753-5131.2009.01006.x

Goda, A. M. A., E. El-Haroun, H. Nazmi, H. Van Doan, A. M. Aboseif, M. K. S. Taha, and N. M. Abou Shabana. 2024. Black soldier fly oil-based diets enriched in lauric acid enhance growth, hematological indices, and fatty acid profiles of Nile tilapia, *Oreochromis niloticus* fry. Aquac. Rep. 37:102269. doi:10.1016/j.aqrep.2024.102269

Guerreiro, I., C. Castro, B. Antunes, F. Coutinho, F. Rangel, A. Couto, ... P. Enes. 2020. Catching black soldier fly for meagre: Growth, whole-body fatty acid profile and metabolic responses. Aquaculture 516:734613. doi:10.1016/j.aquaculture.2019.734613

Hall, G. M. 2010. Fishmeal production and sustainability. p.207–235. *in*: Fish Processing: Sustainability and New Opportunities (Hall, G. M., ed.) Wiley-Blackwell. Chichester, UK. 320 pp. doi:10.1002/9781444328585.ch9

Hasan, I., F. Gai, S. Cirrincione, S. Rimoldi, G. Saroglia, and G. Terova. 2023. Chitinase and insect meal in aquaculture nutrition: A comprehensive overview of the latest achievements. Fishes 8:607. doi:10.3390/fishes8120607

Henry, M., L. Gasco, G. Piccolo, and E. Fountoulaki. 2015. Review on the use of insects in the diet of farmed fish: Past and future. Anim. Feed Sci. Technol. 203:1–22. doi:10.1016/j.anifeedsci.2015.03.001

Hosseindoust, A., S. H. Ha, J. Y. Mun, and J. S. Kim. 2023. Quality characteristics of black soldier flies produced by different substrates. Insects 14:500. doi:10.3390/insects14060500

Hu, Z., M. Xia, G. Wang, L. Jia, H. Ji, J. Sun, and H. Yu. 2025. A superior chitin product: Black soldier fly larvae chitin, beneficial to growth performance, muscle quality and health status of largemouth bass *Micropterus salmoides*, in comparison to shrimp chitin. Aquaculture 595:741667. doi:10.1016/j.aquaculture.2024.741667

Hua, K., J. M. Cobcroft, A. Cole, K. Condon, D. R. Jerry, A. Mangott, ... J. M. Strugnell. 2019. The future of aquatic protein: Implications for protein sources in aquaculture diets. One Earth 1:316–329. doi:10.1016/j.oneear.2019.10.018

Huang, B., S. Zhang, X. Dong, S. Chi, Q. Yang, H. Liu, ... S. Xie. 2022. Effects of fishmeal replacement by black soldier fly on growth performance, digestive enzyme activity, intestine morphology, intestinal flora and immune response of pearl gentian grouper (*Epinephelus fuscoguttatus* ♀ × *Epinephelus lanceolatus* ♂). Fish Shellfish Immunol. 120:497–506. doi:10.1016/j.fsi.2021.12.027

Huang, C., W. Feng, J. Xiong, T. Wang, W. Wang, C. Wang, and F. Yang. 2019. Impact of drying method on the nutritional value of the edible insect protein from black soldier fly (*Hermetia illucens* L.) larvae: Amino acid composition, nutritional value evaluation, *in vitro* digestibility, and thermal properties. Eur. Food Res. Technol. 245:11–21. doi:10.1007/s00217-018-3136-y

Huang, W. C., T. H. Tsai, L. T. Chuang, Y. Y. Li, C. C. Zouboulis, and P. J. Tsai. 2014. Anti-bacterial and anti-inflammatory properties of capric acid against *Propionibacterium acnes*: A comparative study with lauric acid. J. Dermatol. Sci. 73:232–240. doi:10.1016/j.jdermsci.2013.10.010

Hurtado-Ribeira, R., D. M. Hernández, D. Villanueva-Bermejo, M. R. García-Risco, M. D. Hernández, L. Vázquez, ... D. Martin. 2023. The interaction of slaughtering, drying, and defatting methods differently affects oxidative quality of the fat from black soldier fly (*Hermetia illucens*) larvae. Insects 14:368. doi:10.3390/insects14040368

Ido, A., M. F. Z. Ali, T. Takahashi, C. Miura, and T. Miura. 2021. Growth of yellowtail (*Seriola quinqueradiata*) fed on a diet including partially or completely defatted black soldier fly (*Hermetia illucens*) larvae meal. Insects 12:722. doi:10.3390/insects12080722

Isibika, A., B. Vinnerås, O. Kibazohi, C. Zurbrügg, and C. Lalander. 2021. Co-composting of banana peel and orange peel waste with fish waste to improve conversion by black soldier fly (*Hermetia illucens* (L.), Diptera: Stratiomyidae) larvae. J. Clean. Prod. 318:128570. doi:10.1016/j.jclepro.2021.128570

Jannathulla, R., V. Rajaram, R. Kalanjiam, K. Ambasankar, M. Muralidhar, and J. S. Dayal. 2019. Fishmeal availability in the scenarios of climate change: Inevitability of fishmeal replacement in aquafeeds and approaches for the utilization of plant protein sources. Aquac. Res. 50:3493–3506. doi:10.1111/are.14324

Janssen, R. H., J. P. Vincken, L. A. M. van den Broek, V. Fogliano, and C. M. M. Lakemond. 2017. Nitrogen-to-protein conversion factors for three edible insects: *Tenebrio molitor*, *Alphitobius diaperinus*, and *Hermetia illucens*. J. Agric. Food Chem. 65:2275–2278. doi:10.1021/acs.jafc.7b00471

Jobling, M. 2016. Fish nutrition research: Past, present and future. Aquac. Intl. 24:767–786. doi:10.1007/s10499-014-9875-2

Joosten, L., A. Lecocq, A. B. Jensen, O. Haenen, E. Schmitt, and J. Eilenberg. 2020. Review of insect

pathogen risks for the black soldier fly (*Hermetia illucens*) and guidelines for reliable production. Entomol. Exp. Appl. 168:432–447. doi:10.1111/eea.12916

Jorge Iñaki, G. B., P. C. Gerardo Antonio, D. Efrén, M. R. Hiram, G. I. Daniela, and R. J. Damián. 2022. Black soldier fly: Prospection of the inclusion of insect-based ingredients in extruded foods. Food Chem. Adv. 1:100075. doi:10.1016/j.focha.2022.100075

Karapanagiotidis, I. T., M. C. Neofytou, A. Asimaki, E. Daskalopoulou, P. Psofakis, E. Mente, ... C. G. Athanassiou. 2023. Fishmeal replacement by full-fat and defatted *Hermetia illucens* prepupae meal in the diet of gilthead seabream (*Sparus aurata*). Sustainability 15:786. doi:10.3390/su15010786

Kawashima, H. 2019. Intake of arachidonic acid-containing lipids in adult humans: Dietary surveys and clinical trials. Lipids Health Dis. 18:101. doi:10.1186/s12944-019-1039-y

Khieokhajonkhet, A., P. Uanlam, K. Ruttarattanamongkol, N. Aeksiri, P. Tatsapong, and G. Kaneko. 2022. Replacement of fish meal by black soldier fly larvae meal in diet for goldfish *Carassius auratus*: Growth performance, hematology, histology, total carotenoids, and coloration. Aquaculture 561:738618. doi:10.1016/j.aquaculture.2022.738618

Kim, T. K., J. Y. Cha, H. I. Yong, H. W. Jang, S. Jung, and Y. S. Choi. 2022. Application of edible insects as novel protein sources and strategies for improving their processing. Food Sci. Anim. Resour. 42:372–388. doi:10.5851/kosfa.2022.e10

Kirimi, J. G., L. M. Musalia, A. Magana, and J. M. Munguti. 2020. Protein quality of rations for Nile tilapia (*Oreochromis niloticus*) containing oilseed meals. J. Agric. Sci. 12:82–91. doi:10.5539/jas.v12n2p82

Kuo, I. P., C. S. Liu, S. D. Yang, S. H. Liang, Y. F. Hu, and F. H. Nan. 2022. Effects of replacing fishmeal with defatted black soldier fly (*Hermetia illucens* Linnaeus) larvae meal in Japanese eel (*Anguilla japonica*) diet on growth performance, fillet texture, serum biochemical parameters, and intestinal histomorphology. Aquac. Nutr. 2022:1866142. doi:10.1155/2022/1866142

Leming, R. and A. Lember. 2005. Chemical composition of expeller-extracted and cold-pressed canola meal. Agraarteadus 16:103–109.

Lemme, A. and P. Klüber. 2024. Rethinking amino acid nutrition of black soldier fly larvae (*Hermetia illucens*) based on insights from an amino acid reduction trial. Insects 15:862. doi:10.3390/insects15110862

Liland, N. S., I. Biancarosa, P. Araujo, D. Biemans, C. G. Bruckner, R. Waagbø, ... E. J. Lock. 2017. Modulation of nutrient composition of black soldier fly (*Hermetia illucens*) larvae by feeding seaweed-enriched media. PLOS ONE 12:e0183188. doi:10.1371/journal.pone.0183188

Lim, S. J., S. S. Kim, G. Y. Ko, J. W. Song, D. H. Oh, J. D. Kim, ... K. J. Lee. 2011. Fish meal replacement by soybean meal in diets for tiger puffer, *Takifugu rubripes*. Aquaculture 313:165–170. doi:10.1016/j.aquaculture.2011.01.007

Luthada-Raswiswi, R., S. Mukaratirwa, and G. O'Brien. 2021. Animal protein sources as a substitute for fishmeal in aquaculture diets: A systematic review and meta-analysis. Appl. Sci. 11:3854. doi:10.3390/app11093854

Ma, Y., C. Xu, M. Li, H. Chen, R. Ye, G. Zhang, ... Y. Li. 2020. Diet with a high proportion replacement of fishmeal by terrestrial compound protein displayed better farming income and environmental benefits in the carnivorous marine teleost (*Trachinotus ovatus*). Aquac. Rep. 18:100449. doi:10.1016/j.aqrep.2020.100449

Mancini, S., I. Medina, V. Iaconisi, F. Gai, A. Basto, and G. Parisi. 2018. Impact of black soldier fly larvae meal on the chemical and nutritional characteristics of rainbow trout fillets. Animal 12:1672–1681. doi:10.1017/S1751731117003421

Marono, S., G. Piccolo, R. Loponte, C. Di Meo, Y. A. Attia, A. Nizza, and F. Bovera. 2015. *In vitro* crude protein digestibility of *Tenebrio molitor* and *Hermetia illucens* insect meals and its correlation with chemical composition traits. Ital. J. Anim. Sci. 14:3889. doi:10.4081/ijas.2015.3889

Mei, H., C. Li, X. Li, B. Hu, L. Lu, J. K. Tomberlin, and W. Hu. 2022. Characteristics of tylosin and enrofloxacin degradation in swine manure digested by black soldier fly (*Hermetia illucens* L.) larvae. Environ. Pollut. 293:118495. doi:10.1016/j.envpol.2021.118495

Meneguz, M., A. Schiavone, F. Gai, A. Dama, C. Lussiana, M. Renna, and L. Gasco. 2018. Effect of rearing substrate on growth performance, waste reduction efficiency and chemical composition of black soldier fly (*Hermetia illucens*) larvae. J. Sci. Food Agric. 98:5776–5784. doi:10.1002/jsfa.9127

Miner, L. P., J. Fernandez-Bayo, F. Putri, D. Niemeier, H. Bischel, and J. S. VanderGheynst. 2022. Predicting black soldier fly larvae biomass and methionine accumulation using a kinetic model for batch cultivation and improving system performance using semi-batch cultivation. Bioprocess Biosyst. Eng. 45:333–344. doi:10.1007/s00449-021-02663-y

Olsen, R. L. and M. R. Hasan. 2012. A limited supply of fishmeal: Impact on future increases in global aqua-

culture production. Trends Food Sci. 27:120–128. doi:10.1016/j.tifs.2012.06.003

Oonincx, D. G. A. B., S. van Broekhoven, A. van Huis, and J. J. A. van Loon. 2015. Feed conversion, survival and development, and composition of four insect species on diets composed of food by-products. PLOS ONE 10:e0144601. doi:10.1371/journal.pone.0144601

Oteri, M., B. Chiofalo, G. Maricchiolo, G. Toscano, L. Nalbone, V. Lo Presti, and A. R. Di Rosa. 2022. Black soldier fly larvae meal in the diet of gilthead sea bream: Effect on chemical and microbiological quality of filets. Front. Nutr. 9:896552. doi:10.3389/fnut.2022.896552

Pascon, G., G. Cardinaletti, E. Daniso, L. Bruni, M. Messina, G. Parisi, and F. Tulli. 2024. Effect of dietary chitin on growth performance, nutrient utilization, and metabolic response in rainbow trout (*Oncorhynchus mykiss*). Aquac. Rep. 37:102244. doi:10.1016/j.aqrep.2024.102244

Purkayastha, D. and S. Sarkar. 2022. Sustainable waste management using black soldier fly larva: A review. Intl. J. Environ. Sci. Technol. 19:12701–12726. doi:10.1007/s13762-021-03524-7

Purschke, B., R. Scheibelberger, S. Axmann, A. Adler, and H. Jäger. 2017. Impact of substrate contamination with mycotoxins, heavy metals and pesticides on the growth performance and composition of black soldier fly larvae (*Hermetia illucens*) for use in the feed and food value chain. Food Addit. Contam. Part A Chem. Anal. Control Expo. Risk Assess. 34:1410–1420. doi:10.1080/19440049.2017.1299946

Raksasat, R., J. W. Lim, W. Kiatkittipong, K. Kiatkittipong, Y. C. Ho, M. K. Lam, ... C. K. Cheng. 2020. A review of organic waste enrichment for inducing palatability of black soldier fly larvae: Wastes to valuable resources. Environ. Pollut. 267:115488. doi:10.1016/j.envpol.2020.115488

Ravi, H. K., C. Guidou, J. Costil, C. Trespeuch, F. Chemat, and M. A. Vian. 2021. Novel insights on the sustainable wet mode fractionation of black soldier fly larvae (*Hermetia illucens*) into lipids, proteins and chitin. Processes 9:1888. doi:10.3390/pr9111888

Rimoldi, S., C. Ceccotti, F. Brambilla, F. Faccenda, M. Antonini, and G. Terova. 2023. Potential of shrimp waste meal and insect exuviae as sustainable sources of chitin for fish feeds. Aquaculture 567:739256. doi:10.1016/j.aquaculture.2023.739256

Rodrigues, D. P., O. M. C. C. Ameixa, J. A. Vázquez, and R. Calado. 2022. Improving the lipid profile of black soldier fly (*Hermetia illucens*) larvae for marine aquafeeds: Current state of knowledge. Sustainability 14:6472. doi:10.3390/su14116472

Saleh, N. E. 2020. Assessment of sesame meal as a soybean meal replacement in European sea bass (*Dicentrarchus labrax*) diets based on aspects of growth, amino acid profiles, haematology, intestinal and hepatic integrity and macroelement contents. Fish Physiol. Biochem. 46:861–879. doi:10.1007/s10695-019-00756-w

Sealey, W. M., T. G. Gaylord, F. T. Barrows, J. K. Tomberlin, M. A. McGuire, C. Ross, and S. St-Hilaire. 2011. Sensory analysis of rainbow trout, *Oncorhynchus mykiss*, fed enriched black soldier fly prepupae, *Hermetia illucens*. J. World Aquac. Soc. 42:34–45. doi:10.1111/j.1749-7345.2010.00441.x

Shahidul Islam, M. and M. Tanaka. 2004. Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: A review and synthesis. Mar. Pollut. Bull. 48:624–649. doi:10.1016/j.marpolbul.2003.12.004

Shamshina, J. L., P. Berton, and R. D. Rogers. 2019. Advances in functional chitin materials: A review. ACS Sustain. Chem. Eng. 7:6444–6457. doi:10.1021/acssuschemeng.8b06372

Shiau, S. Y. and Y. P. Yu. 1999. Dietary supplementation of chitin and chitosan depresses growth in tilapia, *Oreochromis niloticus* × *O. aureus*. Aquaculture 179:439–446. doi:10.1016/S0044-8486(99)00177-5

Shumo, M., I. M. Osuga, F. M. Khamis, C. M. Tanga, K. K. M. Fiaboe, S. Subramanian, ... C. Borgemeister. 2019. The nutritive value of black soldier fly larvae reared on common organic waste streams in Kenya. Sci. Rep. 9:10110. doi:10.1038/s41598-019-46603-z

Siva Raman, S., L. C. Stringer, N. C. Bruce, and C. S. Chong. 2022. Opportunities, challenges and solutions for black soldier fly larvae-based animal feed production. J. Clean. Prod. 373:133802. doi:10.1016/j.jclepro.2022.133802

Smets, R., J. Claes, and M. Van Der Borght. 2021. On the nitrogen content and a robust nitrogen-to-protein conversion factor of black soldier fly larvae (*Hermetia illucens*). Anal. Bioanal. Chem. 413:6365–6377. doi:10.1007/s00216-021-03595-y

Spranghers, T., M. Ottoboni, C. Klootwijk, A. Ovyn, S. Deboosere, B. De Meulenaer, ... S. De Smet. 2017. Nutritional composition of black soldier fly (*Hermetia illucens*) prepupae reared on different organic waste substrates. J. Sci. Food Agric. 97:2594–2600. doi:10.1002/jsfa.8081

Suo, J., T. Liang, H. Zhang, K. Liu, X. Li, K. Xu, ... S. Yang. 2023. Characteristics of aflatoxin B_1 degradation by *Stenotrophomonas acidaminiphila* and it's combination with black soldier fly larvae. Life 13:234. doi:10.3390/life13010234

Takakuwa, F., R. Tanabe, S. Nomura, T. Inui, S. Yamada, A. Biswas, and H. Tanaka. 2022. Availability of black soldier fly meal as an alternative protein source to fish meal in red sea bream (*Pagrus major*, Temminck & Schlegel) fingerling diets. Aquac. Res. 53:36–49. doi:10.1111/are.15550

Taufek, N. M., H. Muin, A. A. Raji, H. Md Yusof, Z. Alias, and S. A. Razak. 2018. Potential of field crickets meal (*Gryllus bimaculatus*) in the diet of African catfish (*Clarias gariepinus*). J. Appl. Anim. Res. 46:541–546. doi:10.1080/09712119.2017.1357560

Thirunavukkarasar, R., P. Kumar, P. Sardar, N. P. Sahu, V. Harikrishna, K. P. Singha, ... G. Krishna. 2022. Protein-sparing effect of dietary lipid: Changes in growth, nutrient utilization, digestion and IGF-I and IGFBP-I expression of genetically improved farmed tilapia (GIFT), reared in inland ground saline water. Anim. Feed Sci. Technol. 284:115150. doi:10.1016/j.anifeedsci.2021.115150

Twibell, R. G., K. A. Wilson, and P. B. Brown. 2000. Dietary sulfur amino acid requirement of juvenile yellow perch fed the maximum cystine replacement value for methionine. J. Nutr. 130:612–616. doi:10.1093/jn/130.3.612

Ullah, S., J. Zhang, B. Xu, A. F. Tegomo, G. Sagada, L. Zheng, ... Q. Shao. 2022. Effect of dietary supplementation of lauric acid on growth performance, antioxidative capacity, intestinal development and gut microbiota on black sea bream (*Acanthopagrus schlegelii*). PLOS ONE 17:e0262427. doi:10.1371/journal.pone.0262427

Wu, R. A., Q. Ding, L. Yin, X. Chi, N. Sun, R. He, ... Z. Li. 2020. Comparison of the nutritional value of mysore thorn borer (*Anoplophora chinensis*) and mealworm larva (*Tenebrio molitor*): Amino acid, fatty acid, and element profiles. Food Chem. 323:126818. doi:10.1016/j.foodchem.2020.126818

Xin, Y., M. Xu, L. Chen, G. Wang, W. Lu, Z. Liu, ... L. Li. 2024. Effects of different defatting methods of black soldier fly (*Hermetia illucens*) larvae meal on the metabolic energy and nutrient digestibility in young laying hens. Animals 14:2521. doi:10.3390/ani14172521

Xing, S., X. Liang, X. Zhang, A. Oliva-Teles, H. Peres, M. Li, ... M. Xue. 2024. Essential amino acid requirements of fish and crustaceans, a meta-analysis. Rev. Aquac. 16:1069–1086. doi:10.1111/raq.12886

Yamamoto, F. Y., B. A. Suehs, M. Ellis, P. R. Bowles, C. E. Older, M. E. Hume, ... D. M. Gatlin III. 2022. Dietary fishmeal replacement by black soldier fly larvae meals affected red drum (*Sciaenops ocellatus*) production performance and intestinal microbiota depending on what feed substrate the insect larvae were offered. Anim. Feed Sci. Technol. 283:115179. doi:10.1016/j.anifeedsci.2021.115179

Yang, C., S. Ma, F. Li, L. Zheng, J. K. Tomberlin, Z. Yu, ... M. Cai. 2022. Characteristics and mechanisms of ciprofloxacin degradation by black soldier fly larvae combined with associated intestinal microorganisms. Sci. Total Environ. 811:151371. doi:10.1016/j.scitotenv.2021.151371

Yang, Y. H., Y. Y. Wang, Y. Lu, and Q. Z. Li. 2011. Effect of replacing fish meal with soybean meal on growth, feed utilization and nitrogen and phosphorus excretion on rainbow trout (*Oncorhynchus mykiss*). Aquacult. Intl. 19:405–419. doi:10.1007/s10499-010-9359-y

Ytrestøyl, T., M. Bou, C. Dimitriou, G. M. Berge, T. K. Østbye, and B. Ruyter. 2023. Dietary level of the omega-3 fatty acids EPA and DHA influence the flesh pigmentation in Atlantic salmon. Aquac. Nutr. 2023:5528942. doi:10.1155/2023/5528942

Zhao, J., J. Pan, Z. Zhang, Z. Chen, K. Mai, and Y. Zhang. 2023. Fishmeal protein replacement by defatted and full-fat black soldier fly larvae meal in juvenile turbot diet: Effects on the growth performance and intestinal microbiota. Aquacu. Nutr. 2023:8128141. doi:10.1155/2023/8128141

Zhou, Q. C., K. S. Mai, B. P. Tan, and Y. J. Liu. 2005. Partial replacement of fishmeal by soybean meal in diets for juvenile cobia (*Rachycentron canadum*). Aquac. Nutr. 11:175–182. doi:10.1111/j.1365-2095.2005.00335.x

黑水虻 (*Hermetia illucens*) 作為水產養殖永續性蛋白質原料的綜述：營養限制與改善策略

郭裔培 [1,*] 謝易叡 [2] 楊順德 [3]

摘要

郭裔培、謝易叡、楊順德。2025。黑水虻 (*Hermetia illucens*) 作為水產養殖永續性蛋白質原料的綜述：營養限制與改善策略。台灣農業研究 74(3):205–219。

魚粉是水產養殖飼料中的主要蛋白質來源，然而由於捕撈資源的限制，全球魚粉供應量已無法滿足快速成長的水產養殖產業。黑水虻 (*Hermetia illucens*) 具有將不可食用的有機副產物高效率轉化為蛋白質的能力，被認為是一種永續性的魚粉替代蛋白。然而，使用植物性基質餵養的黑水虻常存在營養限制的問題，這些營養缺口可能對魚類的生長表現、免疫反應以及魚肉品質造成負面影響。此外，幾丁質作為昆蟲外殼的主要成分，在魚類飼料中的作用尚有爭議，部分研究指出幾丁質對魚類健康有正面效益，但另有研究發現幾丁質對某些魚種具有抗營養因子的效果。本綜述評估了黑水虻粉在水產養殖飼料中的營養潛力，黑水虻的主要限制性胺基酸包括精胺酸、異亮胺酸、賴胺酸及甲硫胺酸，而主要限制性脂肪酸為 eicosapentaenoic acid (EPA) 與 docosahexaenoic acid (DHA)。為了提升黑水虻幼蟲的營養品質，本文重點分析其蛋白質含量、胺基酸組成、脂肪酸組成及幾丁質的影響，並探討了脫脂處理、基質調控以及去除幾丁質等具發展潛力的改善策略。

關鍵詞：黑水虻、水產養殖、胺基酸、脂肪酸、幾丁質。

投稿日期：2025 年 1 月 20 日；接受日期：2025 年 4 月 6 日。

[*] 通訊作者：ipkuo@mail.ntou.edu.tw

[1] 國立臺灣海洋大學水產養殖學系助理教授。臺灣 基隆市。

[2] 農業部水產試驗所澎湖漁業生物研究中心副研究員。臺灣 澎湖縣。

[3] 農業部水產試驗所淡水養殖研究中心研究員。臺灣 彰化縣。

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):221–235 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0002

Feature Article

Egg Parasitoids as Important Natural Enemies of Auchenorrhynchan Insect Pests in East Asia

Serguei V. Triapitsyn[1,*]

Abstract

Triapitsyn, S. V. 2025. Egg parasitoids as important natural enemies of auchenorrhynchan insect pests in East Asia. J. Taiwan Agric. Res. 74(3):221–235.

Auchenorrhyncha (Hemiptera) contain some of the economically important agricultural pests worldwide, including many invasive ones. The great diversity of some auchenorrhynchan families such as leafhoppers (Cicadellidae) and planthoppers (Delphacidae) corresponds to at least an equal, but often even greater diversity of their natural enemies, particularly egg parasitoids. Whereas during the past 100 years scientists were able to properly identify and study most of the major and some minor auchenorrhynchan pests, research on their egg parasitoids, which are mainly responsible for their natural control, either has been lagging behind significantly or outright nonexistent. Recent developments in the identification of egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) in the four economically important agroecosystems of rice, grapevines, okra, and tea in East Asia are reviewed, along with their host associations, many of which being either newly established, updated, or corrected.

Key words: Leafhopper, Planthopper, Mymaridae, Trichogrammatidae, Biological control.

INTRODUCTION

Auchenorrhyncha (Hemiptera) contain some of economically most important agricultural pests worldwide, including many invasive ones that were established in other parts of the world from Asia and vice versa. Some of the examples of the most recent, notorious auchenorrhynchan invasive species are the spotted lanternfly, *Lycorma delicatula* (White) (Fulgoridae), in North America (whose known egg parasitoids, *Anastatus* spp. (Hymenoptera: Eupelmidae), are polyphagous and thus unlikely to be used in classical biological control programs due to possible non-target effects), the leafhoppers (Cicadellidae) *Orientus ishidae* (Matsumura) in Europe and North America and *Trifida bilobata* Ohara in Europe (all from Asia), and *Thabena brunnifrons* (Bonfils, Attié and Reynaud) (Issidae) in Taiwan (of unknown origin). The great diversity of some auchenorrhynchan families such as Cicadellidae and, to the lesser extent, planthoppers (Delphacidae) corresponds to at least an equal, but often even greater diversity of their natural enemies, particularly egg parasitoids. Whereas during the past 100 years scientists were able to properly identify and study most of the major and some minor auchenorrhynchan pests, research on their egg parasitoids, which are mainly responsible for their natural control, either has been lagging behind significantly or outright nonexistent. That can be explained in part by several factors, such as lack of taxonomic expertise, general poor knowledge of parasitic Hymenoptera biodiversity, predominant use of chemical pesticides in

Received: March 28, 2025; Accepted: May 7, 2025.
[*] Corresponding author, e-mail: serguei.triapitsyn@ucr.edu
[1] Principal Museum Scientist, Department of Entomology, University of California, Riverside, CA, USA.

conventional agriculture and limited nature of organic farming, lack of proper funding, etc. However, in the long perspective there is no alternative to more sustainable agricultural practices that would include biological control as an important part of integrated pest management. Knowledge of the native natural enemies and their correct identification is even more important for the exotic introduced pests beyond their natural distribution range, using classic biological control method.

This review overviews recent developments in the identification of egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of leafhoppers and/or planthoppers in the four economically important agroecosystems of rice, grapevines, okra, and tea in East Asia. Whereas there is abundance of the published literature on egg parasitoids of the auchenorrhynchan rice pests, summarized in Chu & Hirashima (1981), Hirashima (1981), Gurr *et al*. (2011), Lou *et al*. (2013), Triapitsyn (2013), and Sann *et al*. (2018), identities of the listed mymarid and trichogrammatid taxa often have not been verified taxonomically and molecularly and also include many incorrect identifications, synonymized scientific names, and doubtful host association records. These have proven to be either very difficult, or outright impossible, to verify and/or re-identify because historical voucher specimens in insect collections are often missing (particularly primary molecular vouchers of the genetic studies) or are of very poor quality and thus unsuitable for both morphological and molecular identification. The latter also depends heavily on the presence of sequences deposited in public genetic databases that are based on specimens with verified taxonomic identities based on morphology, which are scarce at best. Thus, research had to be done from scratch that would include proper rearing methods from known leafhopper and planthopper hosts (such as sentinel eggs) and reliable taxonomic identifications based both on morphology and molecular studies, including availability and proper deposition of the primary molecular vouchers following non-destructive DNA extraction.

Besides the significant number of published studies on egg parasitoids of auchenorrhynchan pests of rice in East Asia, those on other crops were at most scarce but usually nonexistent. Historically, regional studies of egg parasitoids of rice leafhopper and planthopper pests in East Asia were limited to at most two countries (Hirashima 1981) but were predominantly conducted in one country or region within that country only. Identifications were often made by both non-taxonomists and professional taxonomists in the absence of proper identification keys and thorough generic revisions, this resulting either in misidentifications or descriptions of the same species from several countries. For instance, Pang & Wang (1985) described four new species of *Anagrus* Haliday (Mymaridae) from Guangdong, China, from eggs of rice planthoppers; however, all of these were later synonymized under existing, valid species described from other countries (Triapitsyn 2015). Among them, *A. nilaparvatae* Pang and Wang, the predominant egg parasitoid of the brown planthopper, *Nilaparvata lugens* (Stål), and other rice planthoppers throughout Asia was proven to be a synonym of *A. incarnatus* Haliday (Triapitsyn *et al*. 2018a).

To avoid confusion, only currently valid, named species of egg parasitoids are illustrated below, often with the lists of their main synonyms and the confirmed or presumed auchenorrhynchan hosts.

EGG PARASITOIDS OF RICE LEAFHOPPERS AND PLANTHOPPERS IN EAST ASIA

Surveys of egg parasitoids of rice leafhoppers and planthoppers using sentinel egg method were conducted in Taiwan during 2016–2018 (Triapitsyn *et al*. 2018b, 2020b, 2021b) and, more recently, on Kyushu Island in southern Japan by Hiroki Kusuhara (Kusuhara & Triapitsyn 2023; also H. Kusuhara, personal communication, unpublished data). In East Asia, most of the previous studies were conducted

in mainland China, as summarized by Gurr *et al*. (2011) and Lou *et al*. (2013), Japan (e.g., Vungsilabutr 1978; Yashiro 1979; Miura 1990; Vungsilabutr *et al*. 1996), the Philippines (e.g., Sann *et al*. 2018), South Korea (e.g., Chang 1980; Kim 1984), and Taiwan (e.g., Lin 1974; Miura *et al*. 1981; Chen & Yu 1989; Chen 1993; also summarized in Chu & Hirashima 1981). According to Triapitsyn *et al*. (2018a, 2018b, 2020a, 2021a, 2021b, 2023a) and Kusuhara & Triapitsyn (2023), the main egg parasitoids of rice leafhoppers and planthoppers in East Asia are as follows.

Mymaridae

Anagrus (*Anagrus*) *incarnatus* Haliday (Fig. 1) is by far the most common egg parasitoid of rice planthoppers throughout East and other parts of Asia: *Laodelphax striatella* (Fallén) (small brown planthopper), *Nilaparvata lugens*, and *Sogatella furcifera* (Horváth) (whitebacked planthopper) (Triapitsyn *et al*. 2018b, 2021b). It was also reared from eggs of the rice leafhoppers *Maiestas dorsalis* (Motschulsky) (zig-zag leafhopper) and *Nephotettix cincticeps* (Uhler) (green rice leafhopper) (Triapitsyn *et al*. 2018a, 2018b, 2021b) although its main hosts appear to be various Delphacidae. According to Triapitsyn *et al*. (2021b), this species was misidentified in Japan, the Philippines, and Taiwan as the New World species *Anagrus flaveolus* Waterhouse that does not occur in the Old World (Chu & Hirashima 1981; Miura *et al*. 1981; Zhu *et al*. 2020). The well-known *Anagrus nilaparvatae* Pang and Wang, on which numerous publications exist (primarily in mainland China), was proven to be a synonym of *A*. *incarnatus* based on a molecular study (Triapitsyn *et al*. 2018a).

Anagrus spp.: Other species of *Anagrus* Haliday, such as *A*. (*Anagrus*) *frequens* Perkins, *A*. (*Paranagrus*) *optabilis* (Perkins) (Fig. 2) and *A*. (*Paranagrus*) *perforator* (Perkins) (Fig. 3), were reported from eggs of rice planthoppers in East Asia, but these are probably at most occasional (at least in Taiwan) natural enemies of the three major rice planthopper pests *Laodelphax striatella*, *Nilaparvata lugens*, and *Sogatella furcifera* as their main hosts in paddy fields are likely to

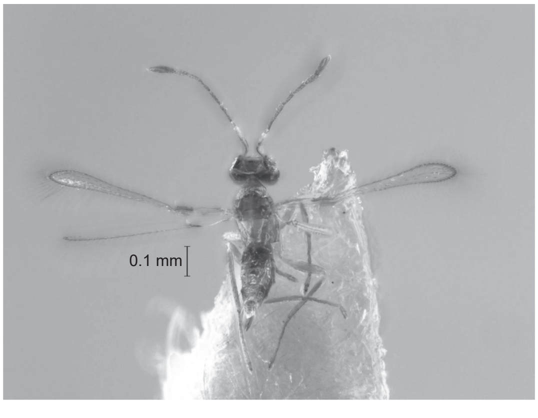

Fig. 1. *Anagrus* (*Anagrus*) *incarnatus* (female).

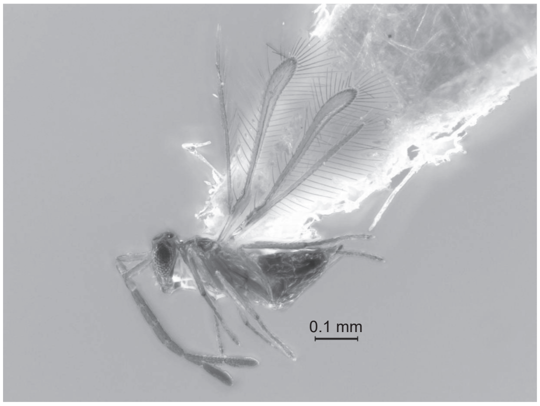

Fig. 2. *Anagrus* (*Paranagrus*) *optabilis* (female).

Fig. 3. *Anagrus* (*Paranagrus*) *perforator* (female).

be other Delphacidae, perhaps also on weeds, for instance *Perkinsiella* sp. for *A. frequens* in Taiwan (Triapitsyn *et al*. 2021b). These three *Anagrus* species can be collected by Malaise and yellow pan traps in rice fields in Japan and Taiwan, with *A. optabilis* being often abundant. However, only one specimen of *A. optabilis* was reared from thousands of sentinel eggs of *Nilaparvata lugens* exposed for parasitization in rice fields in various localities in Taiwan over the three years of surveys (Triapitsyn *et al*. 2021b).

Gonatocerus cincticipitis Sahad (Fig. 4) is a Palaearctic species which so far is known only from Japan and the Republic of Korea (Triapitsyn *et al*. 2023a); it was not reared from eggs of rice leafhoppers in Taiwan (Triapitsyn *et al.* 2021b). It is also likely to occur in the Palearctic part of mainland China and in the southern part of the Russian Far East where rice is cultivated, although there were no published reports of *G. cincticipitis* from there. There was a confusion about its identity (Triapitsyn *et al*. 2021a) that was later resolved by a molecular study (Triapitsyn *et al*. 2023a), since it is morphologically indistinguishable from an undescribed species of *Gonatocerus* Nees ab Esenbeck from Japan. The only known host of *G. cincticipitis* is the green rice leafhopper *Nephotettix cincticeps* (Triapitsyn *et al*. 2021a, 2023a).

Gonatocerus meghalayanus Zeya (Fig. 5) is an Oriental species, originally described from India, that was reared from eggs of the green rice leafhoppers *Nephotettix nigropictus* (Stål) and *N. virescens* (Distant) in the Philippines (Sann *et al*. 2018 [as *G. orientalis* Zeya]) (Triapitsyn *et al*. 2021a). In Taiwan it was collected in a Malaise trap in the organic rice field and misidentified initially as *G. saipanensis* (Triapitsyn *et al*. 2021b [as *G. aegyptiacus*, in part]), where its likely host is *N. nigropictus* (Triapitsyn *et al*. 2021a).

Gonatocerus longicornis Nees ab Esenbeck is present in paddy fields in Japan and Taiwan where its host associations are unknown; elsewhere it is a well-known egg parasitoid of the green leafhopper *Cicadella viridis* (Linnaeus) (Triapitsyn *et al*. 2021b).

Gonatocerus saipanensis (Doutt) (Fig. 6) is a common egg parasitoid of *Nephotettix* spp. green rice leafhoppers, including *N. cincticeps*

Fig. 5. *Gonatocerus meghalayanus* (female).

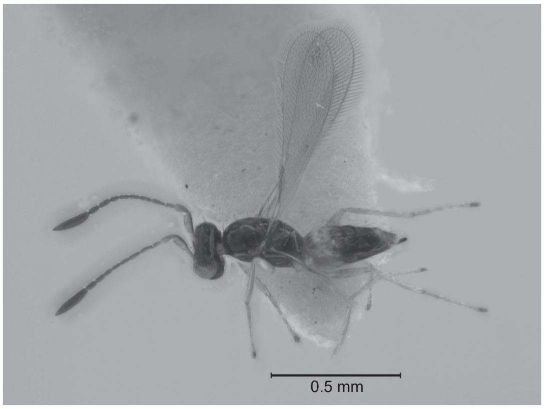

Fig. 4. *Gonatocerus cincticipitis* (female).

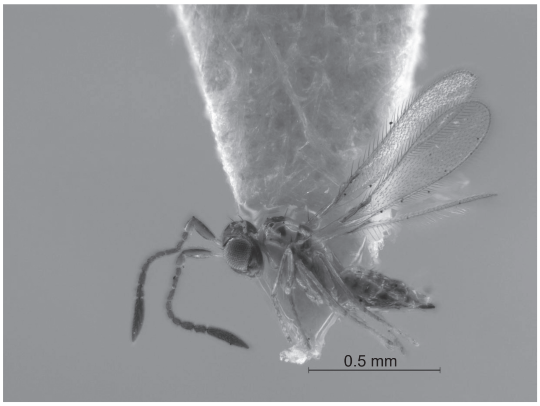

Fig. 6. *Gonatocerus saipanensis* (female).

and *N. virescens*, and it is also known from eggs of *Maiestas dorsalis* (Triapitsyn *et al*. 2021a). It is known from the Palearctic and Oriental regions, Australia, and Oceania, including Japan, Republic of Korea, the Philippines, and Taiwan in East Asia (Triapitsyn *et al*. 2021a). *Gonatocerus saipanensis* was previously misidentified (in part) as *G. aegyptiacus* Soyka (Triapitsyn *et al*. 2018b, 2021b) before the latter was proven to be genetically different (specimens from Egypt) from *G. saipanensis* (specimens from East Asia from paddy fields) (Triapitsyn *et al*. 2023b). Its synonyms include *G. alami* Shamim and Shafee and *G. tarae* (Narayanan and Subba Rao) (both originally described from India), and *G. miurai* Sahad (originally described from Japan) (Triapitsyn *et al*. 2021a). Host records of *G. tarae* from eggs of Delphacidae, such as *Nilaparvata lugens* and *Sogatella furcifera*, need confirmation and are almost certainly erroneous because *G. saipanensis* seems to parasitize only Cicadellidae (Triapitsyn *et al*. 2021b [as *G. aegyptiacus*]).

Two unidentified *Gonatocerus* spp. from the Philippines were reported in Sann *et al*. (2018) from eggs of *Nephotettix* spp. on rice; these could be proven to be undescribed species although they need to be thoroughly compared with all the described, little known species of *Gonatocerus* from South Asia and Australia (Triapitsyn *et al*. 2021a, 2023b).

Lymaenon aureus (Girault) (Fig. 7) was reared in Japan from eggs of zig-zag leafhopper *Maiestas dorsalis* in a rice field (Kusuhara & Triapitsyn 2023).

Lymaenon litoralis (Haliday) (Fig. 8) is a cosmopolitan species, arguably the most common fairyfly on grasses almost worldwide. In East Asia, this species was reared from eggs of zig-zag leafhopper *Maiestas dorsalis* in rice fields in Japan (Kusuhara & Triapitsyn 2023) and Taiwan (Triapitsyn *et al*. 2021b).

Mymar taprobanicum Ward (Fig. 9) is a cosmopolitan species which is a very common and abundant fairyfly in rice fields throughout East Asia, where its leafhopper hosts are *Maiestas dorsalis* and *Nephotettix cincticeps*, whereas its odd record from the brown planthopper *Nilaparvata lugens* in Taiwan (Lin 1974) is almost

Fig. 7. *Lymaenon aureus* (female) (image courtesy of H. Kusuhara).

Fig. 8. *Lymaenon litoralis* (female) (image courtesy of H. Kusuhara).

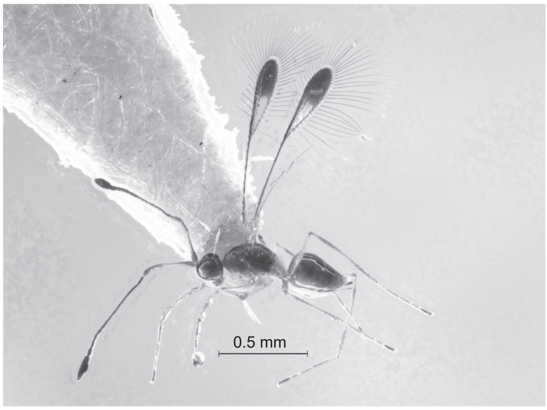

Fig. 9. *Mymar taprobanicum* (female).

certainly erroneous as this parasitoid seems to attack only eggs of Cicadellidae (Triapitsyn *et al*. 2021b).

Trichogrammatidae

Oligosita spp.: Several species were collected from rice fields and reared from eggs of some rice planthoppers throughout East Asia (Yashiro 1979; Lin 1994; Triapitsyn *et al*. 2020b) but their identification remains very problematic because taxonomy of the genus *Oligosita* Walker is in flux. In Japan, *O. japonica* Yashiro (Fig. 10) was collected on rice plants (Yashiro 1979), and in Taiwan presumably this species was reared from eggs of the rice planthoppers *Laodelphax striatella*, *Nilaparvata lugens* and *Sogatella furcifera* (Triapitsyn *et al*. 2020b); it was also reported from eggs of *Nephotettix cincticeps* (Lin 1974) although the latter host record has not been verified. *Oligosita shibuyae* Ishii (Fig. 11), originally described from Japan from specimens that had emerged from rice straw, was also recorded from mainland China and Taiwan by Lin (1994) who indicated *Nephotettix cincticeps* and *Nilaparvata lugens* as its hosts. Yashiro (1979) reported this species from eggs of *Nephotettix cincticeps* in Japan.

Paracentrobia (*Brachistella*) *bicolor* (Girault) (Fig. 12), originally described from Queensland, Australia, is a widespread species throughout East and South Asia and one of the most common egg parasitoids of rice leafhoppers *Nephotettix* spp. and several planthopper species including *Nilaparvata lugens* and *Sogatella furcifera* (Triapitsyn *et al*. 2020b). Its

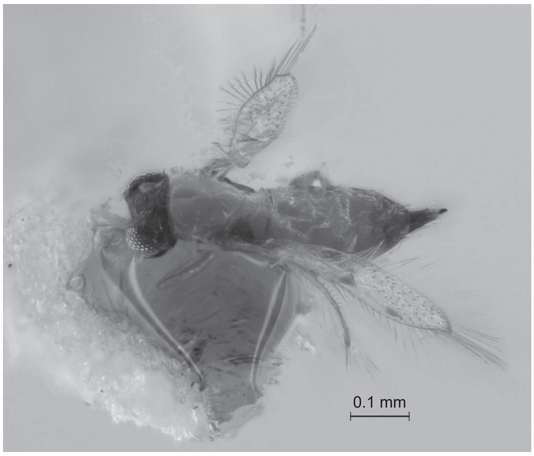

Fig. 10. *Oligosita japonica* (female).

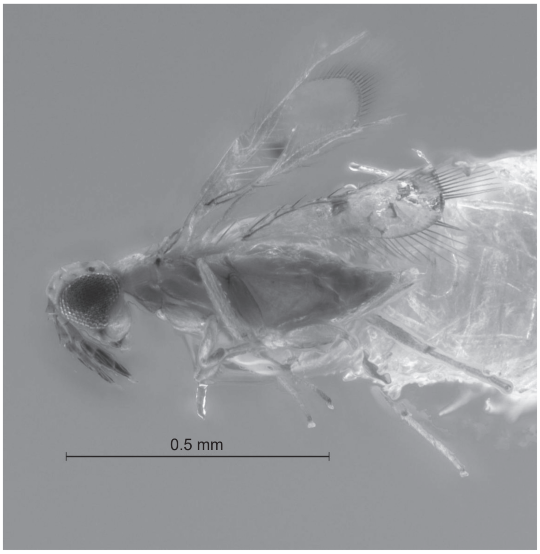

Fig. 11. *Oligosita shibuyae* (female).

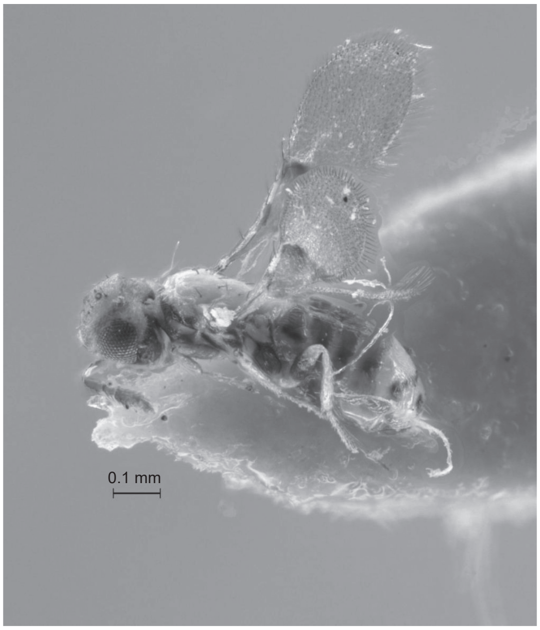

Fig. 12. *Paracentrobia* (*Brachistella*) *bicolor* (female).

synonyms include *Paracentrobia* (*Brachistella*) *andoi* (Ishii), originally described from Japan as *Japania andoi* Ishii, and *Paracentrobia* (*Brachistella*) *garuda* Subba Rao from Thailand (Triapitsyn *et al*. 2020b). Vungsilabutr *et al*. (1996) stated that it was *Paracentrobia yasumatsui* Subba Rao and not *P. andoi* that was definitely parasitic on eggs of *Nilaparvata lugens* in Taiwan, the true *P. andoi* being confirmed in Japan only as a parasitoid of *Nephotettix cincticeps*. Lin (1994) recorded *P. bicolor* (as *P. andoi*) from Fujian and Guangdong, China, also as a parasitoid of *N. cincticeps*.

Paracentrobia (*Paracentrobia*) *mira* (Girault) (Fig. 13), originally described from Queensland, Australia, has a broad distribution in Australasia, Oceania, and the Oriental and eastern Palearctic regions (Triapitsyn 2025). *Paracentrobia mira*, as its synonym *P.* (*Paracentrobia*) *yasumatsui* Subba Rao (originally described from a paddy field in Thailand), was reported from eggs of a number of rice leafhoppers and planthoppers (Lin 1994) but at least some of those records might need to be verified by using a sentinel egg method (Triapitsyn 2025).

Pseudoligosita nephotetticum (Mani) (Fig. 14) is also one of the most common egg parasitoids of rice leafhoppers and planthoppers throughout East Asia, as it is known from the leafhoppers *Maiestas dorsalis*, *Nephotettix cincticeps*, *N. virescens*, and the planthoppers *Laodelphax striatella*, *Nilaparvata lugens*, and *Sogatella furcifera* (Triapitsyn *et al*. 2020b). *Pseudoligosita nephotetticum*, which is known from mainland China, India, Japan, and Taiwan, was also reared from eggs of the leafhoppers *Kolla paulula* (Walker) (Triapitsyn & Shih 2014) and *Amrasca biguttula* (Ishida) (Adachi-Hagimori *et al*. 2023). Its confirmed synonym is *Oligosita tachikawai* Yashiro (originally described from Japan) (Triapitsyn *et al*. 2020b). The Oriental species *Pseudoligosita yasumatsui* (Viggiani and Subba Rao) quite likely is its other synonym (Triapitsyn & Shih 2014) because *P. yasumatsui* only differs from *P. nephotetticum* by a relatively longer ovipositor, and that could possibly be within intraspecific variation of the latter; thus, their genetic comparison is highly desirable.

Discussion

A key to the Mymaridae egg parasitoids (of both sexes) of rice leafhoppers and planthoppers is only available for Taiwan (Triapitsyn *et al*. 2021b), whereas no such keys exist for

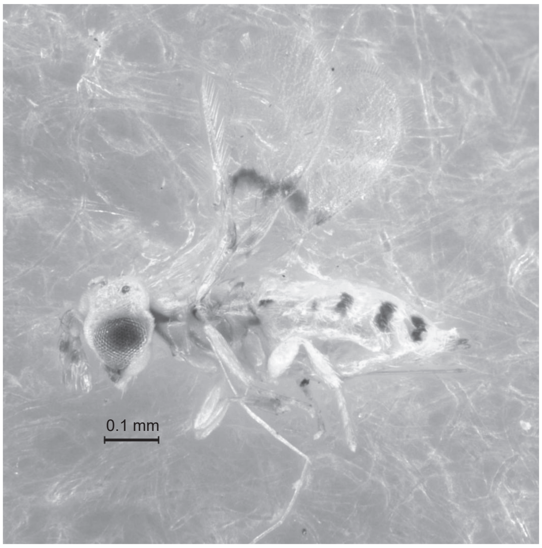

Fig. 13. *Paracentrobia* (*Paracentrobia*) *mira* (female).

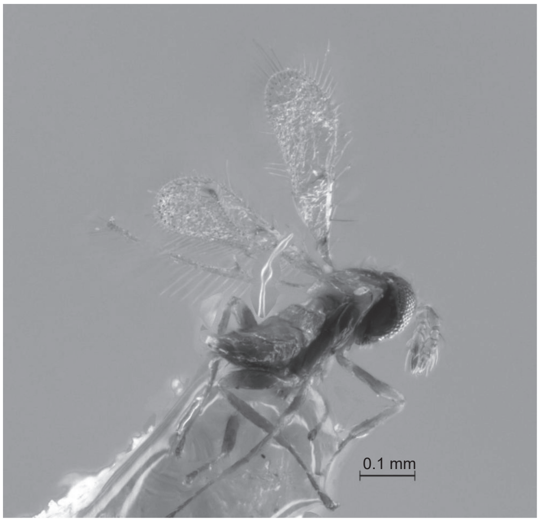

Fig. 14. *Pseudoligosita nephotetticum* (female).

either Mymaridae or Trichogrammatidae for the entire Asian region where rice is cultivated. For the Trichogrammatidae, Triapitsyn *et al*. (2020b) overviewed taxonomy and host associations of such egg parasitoids for Taiwan, but essentially for the entire East Asia, without, however, providing a key to their identification. That makes correct identification of egg parasitoids reared from known rice pest hosts elsewhere often challenging. The aim of this review is to bring together useful information on the known, mostly verified identities of the egg parasitoids of rice leafhoppers and planthoppers in the entire East Asia, and on their trustworthy host associations. This is important because at least 50%, and perhaps up to 70%, of all the previously published records of the egg parasitoids of rice leafhoppers and planthoppers in Asia were apparently either misidentified or used currently outdated scientific names. Incorrect host association records, often resulting from unreliable rearing methods, also contributed to the confusing nature of the historical information on these parasitoids, exacerbated by poor taxonomy of some key genera and multiple descriptions of the same species under different names in single country publications, as well as by lack of regional taxonomic revisions of the key genera and of voucher specimens of the published studies in insect collections.

In summary, most species of Mymaridae and Trichogrammatidae that are main egg parasitoids of rice leafhoppers and planthoppers in the Old World have a wide Pan-Pacific distributions within eastern Palearctic and Oriental regions, and some also within Australasia and Oceania. However, *Gonatocerus cincticipitis* (Mymaridae) is known only from the eastern Palearctic region and *Paracentrobia mira* (Trichogrammatidae) mostly occurs in the Oriental region. Diversity of *Gonatocerus* spp. fairyfly egg parasitoids of *Nephotettix* spp. green rice leafhoppers is the greatest in southern Oriental and Australasian regions where it remains to be thoroughly studied and properly identified. Further synonymies in *Gonatocerus* spp. and some Trichogrammatidae are expected once poorly known Australasian and Oriental region species are revised, particularly in the trichogrammatid genera *Oligosita* and *Pseudoligosita* Girault.

Within each subregion or country, the number and composition of the predominant species of egg parasitoids of rice leafhoppers and planthoppers is quite limited in a given rice field or area and is generally somewhat similar throughout East Asia, while their diversity in southern regions is generally greater than in the northern areas of rice cultivation. However, their abundance and, to some extent, species composition could be expected to vary somewhat from country to country and even within different biogeographical and climatic areas within the same country, such as China and Japan which are situated within two major ecozones (Palearctic and Oriental).

The fairyfly *Anagrus incarnatus* and most Trichogrammatidae parasitize eggs of both Cicadellidae and Delphacidae, whereas *Gonatocerus* spp., *Lymaenon* spp. and *Mymar taprobanicum* (Mymaridae) parasitize only eggs of Cicadellidae on rice plants, so any published records of those from eggs of Delphacidae are likely incorrect due to inaccurate rearing methods. The predominant mymarid egg parasitoids of rice leafhoppers and planthoppers, such as the mymarids *Anagrus incarnatus*, *Gonatocerus saipanensis*, and *Mymar taprobanicum*, and the trichogrammatids *Paracentrobia bicolor* and *Pseudoligosita nephotetticum*, would likely be the same throughout these regions, with local variations.

According to Triapitsyn *et al*. (2021b), in Taiwan *Anagrus incarnatus* was by far the most common and abundant egg parasitoid among both Mymaridae and Trichogrammatidae reared from sentinel eggs of the five-rice leafhopper and planthopper pest species in both the organic and conventional rice fields during four crop seasons in Minxiong Township, Chiayi County. It was a particularly dominant parasitoid of the brown planthopper, *Nilaparvata lugens*, while rarely attacking eggs of the green rice leafhopper

Nephotettix cincticeps. *Gonatocerus saipanensis* and *Mymar taprobanicum* were not discriminating between eggs of the leafhoppers *Maiestas dorsalis* and *Nephotettix cincticeps* in the conventional rice field whereas in the organic rice field *M. taprobanicum* was clearly a dominant egg parasitoid of *M. dorsalis*. In the same survey but for the Trichogrammatidae (Triapitsyn *et al.* 2020b), *Pseudoligosita nephotetticum* was found to be the most common egg parasitoid of the same two leafhopper and three planthopper pests of rice in Taiwan, albeit in much lower overall numbers than *A. incarnatus*.

EGG PARASITOIDS OF THE GRAPE LEAFHOPPER *Arboridia apicalis* (Nawa) ON CULTIVATED GRAPEVINES IN JAPAN AND OF SOME CICADELLINAE IN JAPAN AND TAIWAN

There was no information on egg parasitoids of the leafhoppers feeding on cultivated grapes (*Vitis* spp., Vitaceae) prior to the surveys conducted on Honshu and Kyushu Islands in Japan in 2019 (Triapitsyn *et al.* 2020a), and in 2022 on Honshu and Tanegashima Islands and in 2023 on Honshu Island (S. V. Triapitsyn and T. Adachi-Hagimori, unpublished). While two grape leafhopper species had been previously reported in Japan, *Arboridia* (*Arboridia*) *apicalis* (Nawa) and *A.* (*Arboridia*) *suzukii* (Matsumura), only *A. apicalis* was found during these surveys on Honshu and Kyushu Islands. In a few existing organic vineyards, its eggs were parasitized by *Anagrus* (*Anagrus*) *arboridiae* Triapitsyn and Adachi-Hagimori (Fig. 15), which was described from the specimens reared in 2019 (Triapitsyn *et al.* 2020a). In that study the same authors also reared from eggs of *A. apicalis*, albeit in very low numbers, three trichogrammatid species, *Aphelinoidea* (*Aphelinoidea*) sp., *Oligosita pallida* Kryger, and *Oligosita* sp. As *A. apicalis* and other *Arboridia* spp. also occur, apparently as minor pests, in other countries in East Asia, similar studies of their egg parasitoids there are warranted.

The black-tipped leafhopper *Bothrogonia ferruginea* (Fabricius) is occasionally a minor pest in organic vineyards and kiwifruit orchards in Japan (Triapitsyn & Adachi-Hagimori 2024); it is a known vector of the phytopathogenic bacterium *Xyllella fastidiosa* (Xanthomonadales: Xanthomonadaceae) on cultivated grapes in Taiwan (Tuan *et al.* 2016). Egg parasitoids of *B. ferruginea*, a widespread species in parts of Asia, were previously unknown. Triapitsyn & Adachi-Hagimori (2024) reported *Cosmocomoidea tenuis* Xu, Lin and Hu (Mymaridae) (Fig. 16), originally described from Fujian, China (Aishan *et al.* 2016), as an apparent egg parasitoid of *B. ferruginea* in an organic kiwifruit orchard on Honshu Island in Japan and identified *C. tenuis* as an egg parasitoid of another sharpshooter leafhopper (Cicadellinae: Cicadellini) species, *Kolla paulula*, also a known vector of *X. fastidiosa* in Taiwan (Shih *et al.* 2009). *Cosmocomoidea tenuis* was previously reported (as *Gonatocerus* (*Cosmo-*

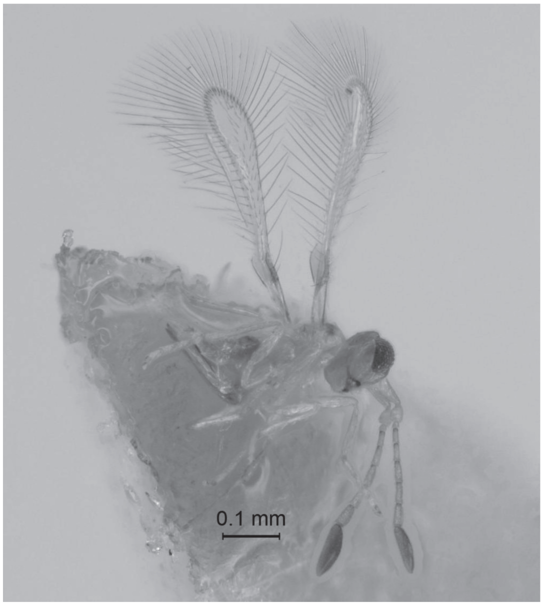

Fig. 15. *Anagrus* (*Anagrus*) *arboridiae* (female).

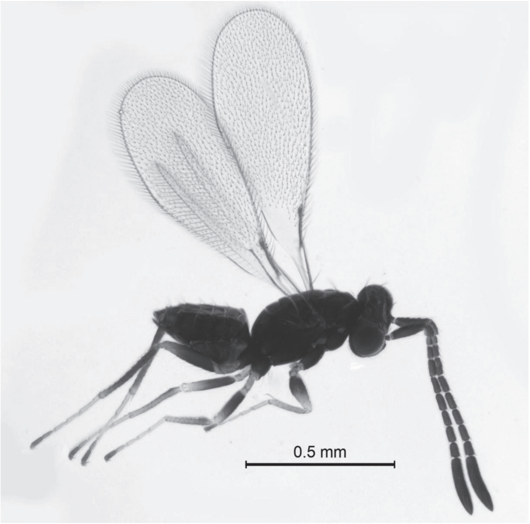

Fig. 16. *Cosmocomoidea tenuis* (female).

comoidea) sp.) from eggs of *K. paulula* in Taiwan along with the trichogrammatid *Pseudoligosita nephotetticum* (Fig. 14) (Triapitsyn & Shih 2014).

EGG PARASITOIDS OF THE OKRA LEAFHOPPER, *Amrasca biguttula* (Ishida), IN JAPAN

The okra leafhopper, *Amrasca* (*Sundapteryx*) *biguttula* (Ishida), is widely distributed across the Oriental region, Micronesia, and parts of the central and eastern Palearctic regions (Xu *et al*. 2017) as an economically important pest of okra, *Abelmoschus esculentus* (Malvaceae), as well as of other crops such as cotton, eggplant, and pepino, and of some ornamental plants such as *Hibiscus* spp. This leafhopper has recently invaded parts of Africa, so knowledge of its natural enemies in its native range in Asia is important for any classical biological control program there. Adachi-Hagimori *et al*. (2020, 2023, 2025) reported the following egg parasitoids of *A. biguttula* on okra in southern Japan (including Ryukyu and Ogasawara Islands): the fairyflies *Anagrus* (*Anagrus*) *atomus* (Linnaeus) (Fig. 17), *Anagrus* (*Anagrus*) *japonicus* Sahad (Fig. 18), *Anagrus* (*Anagrus*) *turpanicus* Triapitsyn and Hu (Fig. 19) (which was previously described in Hu & Triapitsyn (2016) from eggs of an invasive *Arboridia* sp. on table grapes in Xinjiang Uyghur Autonomous Region of China), *Arescon enocki* (Subba Rao and Kaur) (Fig. 20), and *Stethynium empoascae* Subba Rao (Fig. 21), as well as the trichogrammatid *Pseudoligosita nephotetticum* (Fig. 14) on Okinawa Island, Ryukyu Islands only. Adachi-Hagimori *et al*. (2025) also recommended introduction of *Anagrus japonicus* and *Arescon enocki* to the remote Ogasawara Islands from

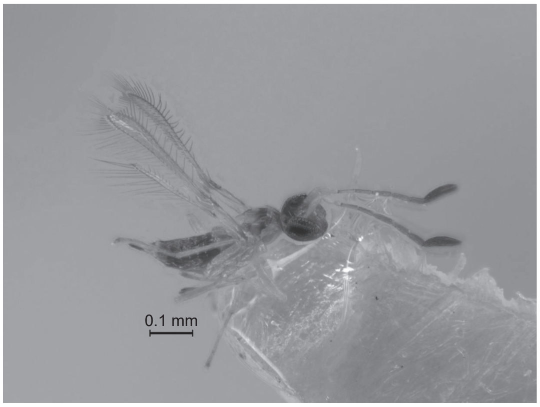

Fig. 17. *Anagrus* (*Anagrus*) *atomus* (female).

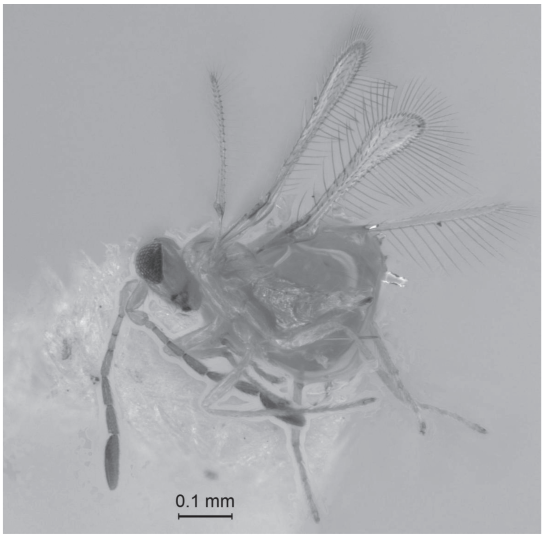

Fig. 18. *Anagrus* (*Anagrus*) *japonicus* (female).

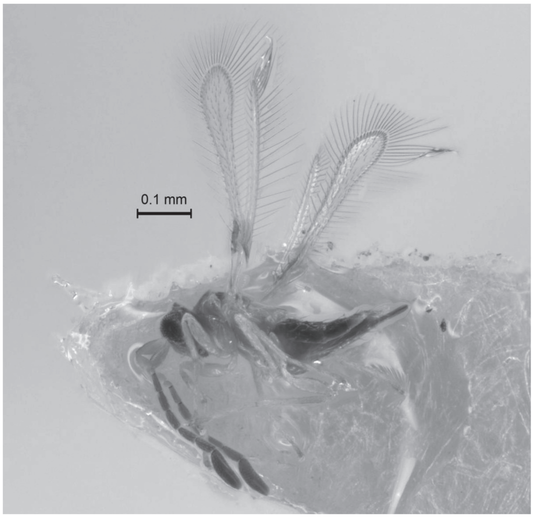

Fig. 19. *Anagrus* (*Anagrus*) *turpanicus* (female).

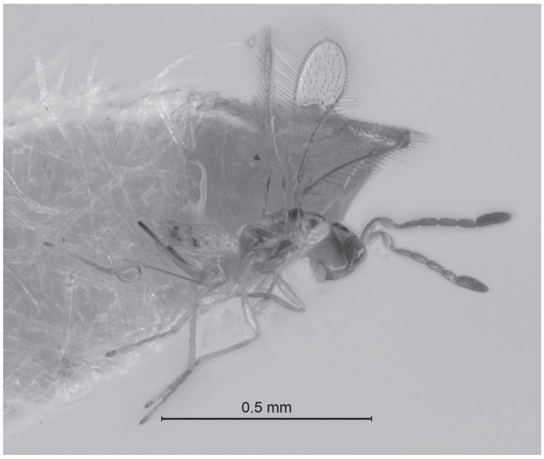

Fig. 20. *Arescon enocki* (female).

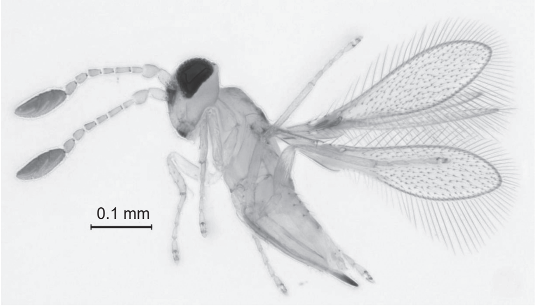

Fig. 21. *Stethynium empoascae* (female).

the Ryukyu Islands as classical biological control agents against *A. biguttula* on okra because only *Stethynium empoascae* was found to be parasitizing eggs of this pest on okra and *Hibiscus* sp. plants on Chichijima Island.

EGG PARASITOIDS OF THE TEA GREEN LEAFHOPPER *Empoasca onukii* (Matsuda) ON TEA PLANTS IN JAPAN

The tea green leafhopper, *Empoasca* (*Matsumurasca*) *onukii* Matsuda, is one of the major pests of tea plants in Japan, mainland China and Taiwan, and it also occurs in Vietnam (Qin *et al*. 2015). Takagi (1978) found fairyflies (Mymaridae) in tea fields in Japan, and Ojima *et al*. (2010) provided data on the population dynamics of three fairyfly species parasitizing eggs of *E. onukii* in tea plantations in Kochi Prefecture, Shikoku Island, Japan, but unfortunately no voucher specimens of their studies exist. *Anagrus* (*Anagrus*) *rugmanjonesi* Triapitsyn and Adachi-Hagimori (Fig. 22) was described from eggs of *E. onukii* on tea plants in Miyazaki Prefecture, Kyushu Island, Japan, in Triapitsyn *et al*. (2019), which likely corresponds to an "?*Anagrus* sp." reported from the same host by Takagi (1978) and to "Mymaridae sp. A (resembling *Anagrus*)" in Ojima *et al*. (2010). Other egg parasitoids of *E. onukii* in Japan, as identified in Triapitsyn *et al*. (2019), are the fairyflies *Arescon enocki* (Fig. 20), which was reported as "Mymaridae sp. C" in Ojima *et al*. (2010), and *Stethynium empoascae* (Fig. 21), which was reported as "Mymaridae sp. B (resembling *Anagrus*)" in Ojima *et al*. (2010). Triapitsyn *et al*. (2019) also recorded *S. empoascae* reared from eggs of *E. onukii* on Kyushu Island.

Because *E. onukii* is a known pest of tea plants in other countries in East Asia (Qin *et al*. 2015), surveys of its egg parasitoids are definitely highly warranted there as well, especially in the organic tea fields where deliberate leafhopper damage to the leaves is believed to achieve an exceptional, unique tea quality.

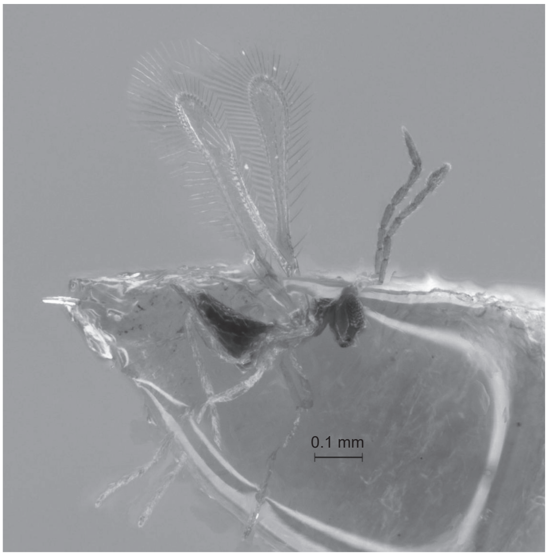

Fig. 22. *Anagrus* (*Anagrus*) *rugmanjonesi* (female).

ACKNOWLEDGMENTS

I thank Drs Hsien-Tzung Shih (Applied Zoology Division, Taiwan Agricultural Research Institute, Wufeng, Taichung, Taiwan), Shou-Horng Huang (Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan), Tetsuya Adachi-Hagimori (Laboratory of Applied Entomology, University of Miyazaki, Miyazaki, Japan) and Hiroki Kusuhara (Laboratory of Insect Natural Enemies, Faculty of Agriculture, Kyushu University, Fukuoka, Japan) for their support and collaboration in the field studies and collection of specimens, and Dr. Paul F. Rugman-Jones (Department of Entomology, University of California, Riverside, California, USA) for multi-year collaboration on the molecular identification of egg parasitoids.

REFERENCES

Adachi-Hagimori, T., S. V. Triapitsyn, and T. Endou. 2025. *Stethynium empoascae* Subba Rao (Hymenoptera: Mymaridae), a newly recorded egg parasitoid of *Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae), a pest of okra on Ogasawara (Bonin) Islands, Japan. J. Asia Pac. Entomol. 28:102379. doi:10.1016/j.aspen.2025.102379

Adachi-Hagimori, T., S. V. Triapitsyn, and P. F. Rugman-Jones. 2023. Egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of okra leafhopper, *Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae), in southern Japan. J. Asia Pac. Entomol. 26:102041. doi:10.1016/j.aspen.2023.102041

Adachi-Hagimori, T., S. V. Triapitsyn, and T. Uesato. 2020. Egg parasitoids (Hymenoptera: Mymaridae) of *Amrasca biguttula* (Ishida) (Hemiptera: Cicadellidae) on Okinawa Island, a pest of okra in Japan. J. Asia Pac. Entomol. 23:791–796. doi:10.1016/j.aspen.2020.07.008

Aishan, Z., S. V. Triapitsyn, M. Xu, N. Q. Lin, and H. Y. Hu. 2016. Review of *Cosmocomoidea* (Hymenoptera: Mymaridae) from China, with descriptions of two new species. Zootaxa 4085(4):525–535. doi:10.11646/zootaxa.4085.4.4

Chang, Y. D. 1980. Egg parasitism of green rice leafhopper, *Nephotettix cincticeps* Uhler by *Gonatocerus* sp. and *Paracentrobia andoi*, in southern rice cultural areas. Korean J. Pl. Prot. 19:109–112. (in Korean with English abstract)

Chen, B. H. 1993. Occurrence and preference of *Anagrus incarnatus* Haliday (Hymenoptera: Mymaridae) on eggs of two rice planthoppers in Taiwan. Plant Prot. Bull. 35:267–275.

Chen, B. H. and J. Z. Yu. 1989. *Anagrus incarnatus* Haliday, a new record from eggs of brown planthopper in Taiwan. J. Agric. Res. China 38:458–462. doi:10.29951/JARC.198912.0008

Chu, Y. I. and Y. Hirashima. 1981. Survey of Taiwanese literature on the natural enemies of rice leafhoppers and planthoppers. Esakia 16:33–37. doi:10.5109/2410

Gurr, G. M., J. Liu, D. M. Y. Read, J. L. A. Catindig, J. A. Cheng, L. P. Lan, and K. L. Heong. 2011. Parasitoids of Asian rice planthopper (Hemiptera: Delphacidae) pests and prospects for enhancing biological control by ecological engineering. Ann. Appl. Biol. 158:149–176. doi:10.1111/j.1744-7348.2010.00455.x

Hirashima, Y. 1981. Field studies on the biological control of leafhoppers and planthoppers (Hemiptera: Homoptera) injurious to rice plants in South-East Asia. An account for the year 1979. Esakia 16:1–4. doi:10.5109/2408

Hu, H.-y. and S. V. Triapitsyn. 2016. *Anagrus turpanicus* sp. n. (Hymenoptera: Mymaridae) from China, an egg parasitoid of *Arboridia kakogowana* [sic] (Hemiptera: Cicadellidae). Zootaxa 4161:573–578. doi:10.11646/zootaxa.4161.4.9

Kim, J. B. 1984. Studies on the egg parasite, *Paracentrobia andoi* Ishii (Hymenoptera: Trichogrammatidae) of green rice leafhopper, *Nephotettix cincticeps* Uhler (1). Korean J. Pl. Prot. 23:237–241. (in Korean with English abstract)

Kusuhara, H. and S. V. Triapitsyn. 2023. A new host record for the leafhopper egg parasitoid *Lymaenon aureus* (Girault, 1911) (Hymenoptera: Mymaridae). Jpn. J. Entomol. (N. Ser.) 26:8–11. doi:10.20848/kontyu.26.1_8

Lin, K. S. 1974. Notes on some natural enemies of *Nephotettix cincticeps* (Uhler) and *Nilaparvata lugens* (Stål) in Taiwan. J. Taiwan Agric. Res. 23:91–115. (in Chinese with English abstract). doi:10.29951/JARC.197406.0002

Lin, N. 1994. Systematic Studies of Chinese Trichogrammatidae (Hymenoptera: Chalcidoidea). Contribution of the Biological Control Research Institute, Fujian Agricultural University, Special Publication No. 4. Fujian Science and Technology Publishing House. Fujian, China. 362 pp. (in Chinese with English abstract)

Lou, Y. G., G. R. Zhang, W. Q. Zhang, Y. Hu, and J. Zhang. 2013. Biological control of rice insect pests in China. Biol. Control 67:8–20. doi:10.1016/j.biocontrol.2013.06.011

Miura, K. 1990. Life-history parameters of *Paracentrobia andoi* (Ishii) (Hymenoptera, Trichogrammatidae), an egg parasitoid of the green rice leafhopper, *Nephotettix cincticeps* Uhler (Homoptera, Deltocephalidae). Jpn. J. Entomol. 58:585–591.

Miura, T., Y. Hirashima, M. T. Chûjô, and Y. I. Chu. 1981. Egg and nymphal parasites of rice leafhoppers and planthoppers. A result of field studies in Taiwan in 1979 (Part 1). Esakia 16:39–50. doi:10.5109/2411

Ojima, I., S. Kotani, and R. Arakawa. 2010. Occurrence of parasitoids of tea green leafhopper on tea plants. Bull. Kochi Agric. Res. Cent. 19:37–44. (in Japanese with English abstract)

Pang, X. F. and Y. Wang. 1985. New species of *Anagrus* from south China (Hymenoptera: Mymaridae). Entomotaxonomia 7:175–184. (in Chinese with English abstract)

Qin, D., L. Zhang, Q. Xiao, C. Dietrich, and M. Matsumura. 2015. Clarification of the identity of the tea green leafhopper based on morphological comparison between Chinese and Japanese specimens. PLOS ONE 10:e0139202. doi:10.1371/journal.pone.0139202

Sann, C., F. Wemheuer, A. Beaurepaire, R. Daniel, S. Erler, and S. Vidal. 2018. Preliminary investigation of species diversity of rice hopper parasitoids in Southeast Asia. Insects 9:19. doi:10.3390/insects9010019

Shih, H. T., C. C. Su, C. Y. Feng, C. C. Fanjiang, W. F. Hung, and L. Y. Hung. 2009. Studies on the morphology, ecology, and host range for *Kolla paulula* (Walker, 1858) (Hemiptera: Membracoidea: Cicadellidae: Cicadellinae). Formosan Entomol. 29:353. (in Chinese with English abstract)

Takagi, K. 1978. Trap for monitoring adult parasites of the tea pest. Jpn. Agric. Res. Q. 12:99–103.

Triapitsyn, S. V. 2013. Taxonomy and biology of egg parasitoids of Auchenorrhyncha of economic importance in Taiwan and adjacent countries, and of proconiine sharpshooters in the New World. p.123–144. *in*: Proceedings of the 2013 International Symposium on Insect Vectors and Insect-Borne Diseases. August 6–8, 2013. Taichung, Taiwan. Taiwan Agric. Res. Inst. Pub. No. 173. Taichung, Taiwan.

Triapitsyn, S. V. 2015. Taxonomy of the genus *Anagrus* Haliday (Hymenoptera: Mymaridae) of the world: An annotated key to the described species, discussion of the remaining problems, and a checklist. Acta Zool. Lilloana 59:3–50. (in English with Spanish abstract)

Triapitsyn, S. V. 2025. Redescription of *Paracentrobia (Paracentrobia) mira* (Girault) (Hymenoptera: Trichogrammatidae), with two new synonyms. J. Asia Pac. Biodivers. 18:118–124. doi:10.1016/j.japb.2024.08.003

Triapitsyn, S. V. and T. Adachi-Hagimori. 2024. A new record of the fairyfly *Cosmocomoidea tenuis* (Hymenoptera: Mymaridae) from Japan, with notes on its host associations. Appl. Entomol. Zool. 59:279–285. doi:10.1007/s13355-024-00877-6

Triapitsyn, S. V., T. Adachi-Hagimori, P. F. Rugman-Jones, A. Barry, A. Abe, K. Matsuo, and K. Ohno. 2019. Egg parasitoids of the tea green leafhopper *Empoasca onukii* (Hemiptera, Cicadellidae) in Japan, with description of a new species of *Anagrus* (Hymenoptera, Mymaridae). ZooKeys 836:93–112. doi:10.3897/zookeys.836.32634

Triapitsyn, S. V., T. Adachi-Hagimori, P. F. Rugman-Jones, N. Kado, N. Sawamura, and Y. Narai. 2020a. Egg parasitoids of *Arboridia apicalis* (Nawa, 1913) (Hemiptera, Cicadellidae), a leafhopper pest of grapevines in Japan, with description of a new species of *Anagrus* Haliday, 1833 (Hymenoptera, Mymaridae). ZooKeys 945:129–152. doi:10.3897/zookeys.945.51865

Triapitsyn, S. V., H. Kusuhara, P. F. Rugman-Jones, and T. Adachi-Hagimori. 2023a. Revised molecular characterization of *Gonatocerus cincticipitis* Sahad (Hymenoptera: Mymaridae), an egg parasitoid of green rice leafhopper *Nephotettix cincticeps* (Uhler) (Hemiptera: Cicadellidae) in Japan. J. Asia Pac. En-

tomol. 26:102069. doi:10.1016/j.aspen.2023.102069

Triapitsyn, S. V., P. F. Rugman-Jones, M. I. Abul-Sood, and N. S. Gadallah. 2023b. On the genetic identity of *Gonatocerus aegyptiacus* (Hymenoptera Mymaridae) from Egypt. Bull. Insectology 76:127–132.

Triapitsyn, S. V., P. F. Rugman-Jones, and T. Adachi-Hagimori. 2021a. Molecular and morphological differentiation within the *Gonatocerus fuscicornis* species complex (Hymenoptera Mymaridae). Bull. Insectology 74:181–200.

Triapitsyn, S. V., P. F. Rugman-Jones, P. S. Tretiakov, H. T. Shih, and S. H. Huang. 2018a. New synonymies in the *Anagrus incarnatus* Haliday 'species complex' (Hymenoptera: Mymaridae) including a common parasitoid of economically important planthopper (Hemiptera: Delphacidae) pests of rice in Asia. J. Nat. Hist. 52:2795–2822. doi:10.1080/00222933.2018.1552333

Triapitsyn, S. V. and H. T. Shih. 2014. Egg parasitoids (Hymenoptera: Mymaridae and Trichogrammatidae) of *Kolla paulula* (Walker) (Hemiptera: Cicadellidae) in Taiwan. J. Asia Pac. Entomol. 17:673–678. doi:10.1016/j.aspen.2014.06.010

Triapitsyn, S. V., H. T. Shih, and S. H. Huang. 2018b. Identification of the egg parasitoids of Auchenorrhyncha (Hemiptera) of economic importance in Taiwan: Collaborative research between Taiwan Agricultural Research Institute and University of California at Riverside scientists. p.4–16. *in*: Proceedings of the 2018 International Symposium on Proactive Technologies for Enhancement of Integrated Pest Management of Key Crops. September 4–6, 2018. Taichung, Taiwan. Taiwan Agric. Res. Inst. Pub. No. 215. Taichung, Taiwan.

Triapitsyn, S. V., H. T. Shih, and S. H. Huang. 2020b. Identification of egg parasitoids of rice leafhoppers and planthoppers (Hemiptera: Cicadellidae and Delphacidae) of economic importance in Taiwan, part 2: Trichogrammatidae (Hymenoptera). J. Asia Pac. Entomol. 23:970–980. doi:10.1016/j.aspen.2020.08.004

Triapitsyn, S. V., H. T. Shih, S. H. Huang, and M. J. Tseng. 2021b. Identification of egg parasitoids of rice leafhoppers and planthoppers (Hemiptera: Cicadellidae and Delphacidae) of economic importance in Taiwan, part 1: Mymaridae (Hymenoptera). J. Asia Pac. Entomol. 24:77–90. doi:10.1016/j.aspen.2020.11.002

Tuan, S. J., F. T. Hu, H. Y. Chang, P. W. Chang, Y. H. Chen, and T. P. Huang. 2016. *Xylella fastidiosa* transmission and life history of two Cicadellinae sharpshooters, *Kolla paulula* and *Bothrogonia ferruginea* (Hemiptera: Cicadellidae), in Taiwan. J. Econ. Entomol. 109:1034–1040. doi:10.1093/jee/tow016

Vungsilabutr, P. 1978. Biological and morphological studies of *Paracentrobia andoi* (Ishii) (Hymenoptera: Trichogrammatidae), a parasite of the green rice leafhopper, *Nephotettix cincticeps* Uhler (Homoptera: Deltocephalidae). Esakia 11:29–51. doi:10.5109/2371

Vungsilabutr, P., T. Miura, and K. Miura. 1996. Can *Paracentrobia andoi* Ishii (Hymenoptera, Trichogrammatidae) parasitize the egg of the brown planthopper? Jpn. J. Entomol. 64:876–878.

Xu, Y., Y. Wang, C. H. Dietrich, M. J. Fletcher, and D. Qin. 2017. Review of Chinese species of the leafhopper genus *Amrasca* Ghauri (Hemiptera, Cicadellidae, Typhlocybinae), with description of a new species, species checklist and notes on the identity of the Indian cotton leafhopper. Zootaxa 4353(2):360–370. doi:10.11646/zootaxa.4353.2.7

Yashiro, N. 1979. Studies on the Japanese species of *Oligosita* (Hymenoptera: Trichogrammatidae). Trans. Shikoku Entomol. Soc. 14:195–203.

Zhu, P., X. Zheng, H. Xu, A. C. Johnson, K. L. Heong, G. M. Gurr, and Z. Lu. 2020. Nitrogen fertilizer promotes the rice pest *Nilaparvata lugens* via impaired natural enemy, *Anagrus flaveolus*, performance. J. Pest Sci. 93:757–766. doi:10.1007/s10340-019-01177-7

頸喙亞目昆蟲的重要卵寄生蜂天敵

Serguei V. Triapitsyn[1,*]

摘要

Triapitsyn, S. V. 2025。頸喙亞目昆蟲的重要卵寄生蜂天敵。台灣農業研究 74(3):221–235。

半翅目之頸喙亞目 (Auchenorrhyncha) 昆蟲中含有一些對世界各地而言，屬於重要經濟性的農業害蟲，其中包括許多入侵性害蟲。一些如葉蟬科 (Cicadellidae) 與稻蝨科 (Delphacidae) 這類頸喙亞目昆蟲，其豐富的物種多樣性對應著至少相等或是更多樣性的天敵昆蟲，特別是卵寄生性這類天敵。儘管在過去 100 年中，科學家已經能夠正確識別與研究頸喙亞目當中大多數的主要與次要害蟲，但對於可自然防治這類害蟲的卵寄生蜂相關研究卻已嚴重落後，甚至缺乏研究。最近有關東亞 4 大經濟重要農業生態系統 (稻米、葡萄、秋葵及茶) 中之膜翅目卵寄生蜂，包括纓小蜂科 (Mymaridae) 與赤眼蜂科 (Trichogrammatidae) 的最新進展，將於本文進行回顧綜述，同時說明它們的寄主關聯，其中許多寄主關聯資料為新確立、更新或已被修正。

關鍵詞：葉蟬、稻蝨、纓小蜂科、赤眼蜂科、生物防治。

投稿日期：2025 年 3 月 28 日；接受日期：2025 年 5 月 7 日。
[*] 通訊作者：serguei.triapitsyn@ucr.edu
[1] 美國加州大學昆蟲學系首席博物館科學家。美國 加州。

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):237–248 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0003

Feature Article

Breeding Methods for Improving the Resistance of *Apis mellifera* to *Varroa* Mite (*Varroa destructor*)

Pen-Han Chen[1,*], Zin-Hao Huang[1], Pei-Shou Hsu[2], Chi-Yen Pan[3], and Tzu-Hsien Wu[4]

Abstract

Chen, P. H., Z. H. Huang, P. S. Hsu, C. Y. Pan, and T. H. Wu. 2025. Breeding methods for improving the resistance of *Apis mellifera* to *Varroa* mite (*Varroa destructor*). J. Taiwan Agric. Res. 74(3):237–248.

The Western honey bee (*Apis mellifera* L.) is an essential pollinator and contributes significantly to apiculture. Its health is crucial for food security and agricultural profitability. *Varroa destructor*, an ectoparasitic mite, poses the greatest threat to honey bee health by transmitting pathogens and disrupting development, which negatively affects agricultural productivity. Beekeepers used to control *Varroa* mites with miticides, but prolonged use has led to mite resistance and miticide residues in honey bee products. In addition to chemical control, social immune behaviors of honey bees, such as worker hygienic behavior can enhance the *Varroa* mite management by reducing pathogen and mite reproduction. This review explores the methods for breeding *Varroa* mite-resistant honey bees by: (1) assessing the health status of source colonies; (2) evaluating social immune behavioral competencies and gene expressions for selecting parental colonies; and (3) assessing the performance of social immune traits in progeny colonies. This breeding program will help improve Integrated Pest Management (IPM) strategies.

Key words: *Apis mellifera*, *Varroa destructor*, Social immune behaviors, Integrated pest management.

INTRODUCTION

Western honey bees (*Apis mellifera* L.), the primary species in global apiculture, produce honey, beeswax, bee pollen, and royal jelly. The Food and Agriculture Organization (FAO 2023) estimated that the value of honey and associated products exceeded USD 1,200 billion in 2023. Porto *et al*. (2020) valued eco-pollination services at USD 267–657 billion annually, with bees being the main pollinators crucial for food production and ecosystem sustainability (Hung *et al*. 2018; Khalifa *et al*. 2021). Bee-pollinated crops account for about one-third of the human diet and enhance both the quality and quantity of agricultural products such as coffee, cocoa, and almonds (Stein *et al*. 2017; Khalifa *et al*. 2021).

Honey bees are vital for human well-being and agriculture. However, their health is threatened by multiple factors, including ecto-

Received: March 4, 2025; Accepted: May 19, 2025.
* Corresponding author, e-mail: BanHen@mdares.gov.tw
1 Assistant Research Fellows, Sericulture and Apiculture Division, Miaoli District Agricultural Research and Extension Station, Miaoli County, Taiwan, ROC.
2 Associate Research Fellow, Sericulture and Apiculture Division, Miaoli District Agricultural Research and Extension Station, Miaoli County, Taiwan, ROC.
3 Technical Associate, Sericulture and Apiculture Division, Miaoli District Agricultural Research and Extension Station, Miaoli County, Taiwan, ROC.
4 Research Fellow, Sericulture and Apiculture Division, Miaoli District Agricultural Research and Extension Station, Miaoli County, Taiwan, ROC.

parasites, pathogens, environmental conditions, beekeeper management, nutrition, and pesticide exposure (Johnson *et al*. 2010a; Olate-Olave *et al*. 2021; Ricigliano *et al*. 2022). The ectoparasite mite *Varroa destructor* (*Varroa* mite) feeds on the fat body and haemolymph of adult and larval honey bees, causing weight loss, shortened lifespan, and damage to reproductive ability. *Varroa* mites also transmit viruses that cause wing deformation in adult workers, posing a major threat to the beekeeping industry (Duay *et al*. 2002; Ritter 2006; Ramsey *et al*. 2019; Piou *et al*. 2022). Beekeepers often use miticides, such as synthetic pyrethroid compounds, to control *Varroa* mites. However, overuse has led to resistance in *Varroa* populations, resulting in reducing treatment effectiveness and complicating mite control, which can contribute to colony decline and reduced profitability (Johnson *et al*. 2010b; Avalos *et al*. 2024). Integrated Pest Management (IPM) approaches have been advocated in progressive agricultural countries to control *Varroa* mites. These approaches are based on pest and host biology and incorporate sustainable pest management techniques, including monitoring, risk prediction, control methods, pesticide toxicology, breeding programs, and regulatory management (van Alphen & Fernhout 2020; Jack & Ellis 2021).

Honey bees are eusocial insects and possess social immunity against pathogens and parasites. Social immunity includes behavioral, physiological, and organizational adaptations that reduce pathogen transmission within a colony (Cremer *et al*. 2007). To combat *Varroa* mites and associated pathogens, honey bees have developed hygienic and grooming behaviors. Hygienic behavior involves worker bees detecting and removing diseased, dead, or *Varroa* mite-parasitized brood from the colony. Grooming behavior involves bees using their mandibles and legs to remove *Varroa* mites from their bodies, sometimes attacking or killing them (Gilliam *et al*. 1983; Spivak & Reuter 2001; Evans *et al*. 2006; Morfin *et al*. 2021). These hygiene and grooming behaviors are heritable social immune responses that confer disease resistance in honey bee colonies (Spivak & Reuter 2001; Morfin *et al*. 2021). As mentioned above, breeding programs to improve *Varroa* mite resistance through social immunity are a next important step in IPM strategies. Beekeepers primarily maintain colonies for economic purposes and commonly use chemical treatments to control *Varroa* mites. While this approach has long been practiced in conventional farming, few have leveraged honey bees' social immunity. This review explores the breeding potential of social immunity to enhance *Varroa* mite resistance.

INTEGRATED PEST MANAGEMENT (IPM)

IPM for *Varroa* mites involves bee and mite biology, local regulations, and cooperation with beekeepers and the government. It uses pest population monitoring to establish control thresholds and evaluate treatment effectiveness. The life cycle of the *Varroa* mite consists of two phases: The phoretic (on adult bees) and the reproductive phase (within capped brood cells). Female mites prefer drone brood due to its longer development period, which allows for increased progeny production (Rosenkranz *et al*. 2010; Torres & Torres 2020).

The *Varroa* mite population monitoring measures can be divided into screening for mites on adult bees and detecting mites parasitizing capped brood cells. To monitor *Varroa* mites on adult bees, approximately 300 bees are mixed with powdered sugar, ethanol, or other chemicals, gently shaken to remove the mites, then sieved and counted to determine the parasitism rate per 100 bees (Rinderer *et al*. 2004; Dietemann *et al*. 2013). Compared to ethanol or chemical treatments, the sugar dusting method is gentler on bees. However, chemical treatments are more precise because they ensure the complete removal of all mites. Another method for counting *Varroa* mites is the sticky board

method, in which a sticky board is placed underneath a hive and separated from the bees by a layer of screen mesh. The number of fallen mites on the board can be counted regularly (Delaplane *et al*. 2005). Furthermore, parasite infection in brood cells is mostly assessed by removing drone larvae, because adult female mites prefer to parasitize drones, which often provide sufficient development energy for *Varroa* mite nymphs (Dietemann *et al*. 2013).

Monitoring measures can also be used to determine control thresholds for *Varroa* mites and the effectiveness of treatments. The threshold for *Varroa* mite population is set below the economic loss level, as the pest population continues to grow until treated. Various factors, including seasonal variation, geographic region, monitoring method, virus prevalence, and honeybee genetics, influence the determination of these thresholds. For instance, Morfin *et al*. (2024) reported that a *Varroa* mite infestation rate of 1% or higher, monitored through ethanol washing, resulted in higher colony mortality in the following spring compared to colonies with lower mite infestation rates in the fall in Canada. Similarly, Currie & Gatien (2006) established a 2% threshold for *Varroa* mite control on adult bees in the Canadian prairie region to prevent honey production losses. Additionally, Delaplane *et al*. (2005) found that colonies with the suppressed mite reproduction trait took longer to reach the control threshold compared to conventional colonies.

Varroa mite control treatments include physical and chemical methods. When *Varroa* mite levels are below the control threshold, physical treatments such as removing drone brood during inspections and using sticky boards on the bottom of the hive can help reduce mite reproduction (Rosenkranz *et al*. 2010; Dietemann *et al*. 2013). For mite populations that reach the control threshold, chemical treatments like synthetic pyrethroids (e.g. tau-fluvalinate and flumethrin) are recommended. However, resistance to these chemicals has been reported in various countries. Therefore, alternative miticides or organic acids are suggested for IPM strategies (Martin 2004; González-Cabrera *et al*. 2016).

In addition to human interventions, Spivak & Reuter (2001) observed that honey bee colonies exhibit social immunity by removing brood pathogens, thereby enhancing disease resistance. Morfin *et al*. (2021) further noted that hygienic behavior can improve a colony's resistance to *Varroa* mites. As a heritable social immune response in honey bees, hygienic behavior can serve as a trait for breeding healthy colonies, thereby increasing the efficiency of *Varroa* mite management.

SOCIAL IMMUNITY OF WESTERN HONEY BEE

Western honey bee is a eusocial insect that employs various defensive mechanisms against pathogens and parasites. These mechanisms include the innate immunity of the individual bees and the social immunity of the colony. Social immunity represents the collective efforts of individuals to limit the spread of parasites and pathogens, thereby protecting uninfected bees. Examples of social immunity of honey bees against *Varroa* mites include grooming and hygienic behaviors (Spivak & Reuter 2001; Harpur *et al*. 2019). Grooming behavior involves honey bees using their mandibles and legs to remove *Varroa* mites from their bodies. Invernizzi *et al*. (2015) found that Africanized honey bees were more successful at dislodging *Varroa* mites (65.9 ± 15.6%) than Italian honey bees (60.8 ± 20.0%) in petri dish tests, though the difference was not statistically significant. Additionally, Africanized honey bees exhibited a significantly higher tendency to injure *Varroa* mites (29.0 ± 8.6%) than Italian bees (17.7 ± 9.8%). Another study by Morfin *et al*. (2020) compared the expressions of *AmNrx-1* (neurexin) between Indiana mite-biter colonies and unselected Italian colonies. The Indiana mite-biter colonies showed higher *AmNrx-1* expression, a

greater proportion of mutilated mites and higher winter survival rates compared to unselected Italian colonies.

Hygienic behavior in bees refers to the ability of worker bees to detect and remove infected broods, as well as dead or unhealthy bees from the hive. Field studies have demonstrated that such behavior significantly reduces the prevalence of disease such as chalkbrood and American foulbrood, as well as the parasitic mite *V. destructor* (Gilliam *et al.* 1983; Spivak & Reuter 2001; Harbo & Harris 2009). The assessment of hygienic behavior can be conducted through the pin-killed brood test and the liquid nitrogen-killed brood test (Fig. 1). These methods involve killing 100–300 capped cells containing young pupae either by piercing the caps or by applying liquid nitrogen. After returning the treated frames to the hive for 12–30 h, the removal rate of the killed pupae is evaluated to determine the colony's hygienic behavior efficiency (Fig. 1A) (Büchler *et al.* 2013).

Masaquiza *et al.* (2021) reported that hygienic bee colonies exhibited a lower *Varroa* mite infestation rate on adult bees (3.47 ± 1.56%) and achieved the highest honey production (25.08 ± 4.82 kg hive^{-1}) compared to control colonies. Hawkins & Martin (2021) demonstrated that in hygienic colonies, approximately 40% capped cells artificially infested with *Varroa* mite were removed, which was higher than in control colonies. Additionally, the reproductive efficiency of *Varroa* mite in hygienic colonies was lower than in control colonies. Similar results were reported by Khan & Ghramh (2021); in hygienic colonies, the infestation rate of artificially infected *Varroa* mites in capped cells was (10.28 ± 0.86%), significantly lower than that of control colonies (22.78 ± 1.41%). These findings indicate that hygienic colonies possess a superior ability to detect and remove *Varroa* mite-infested brood.

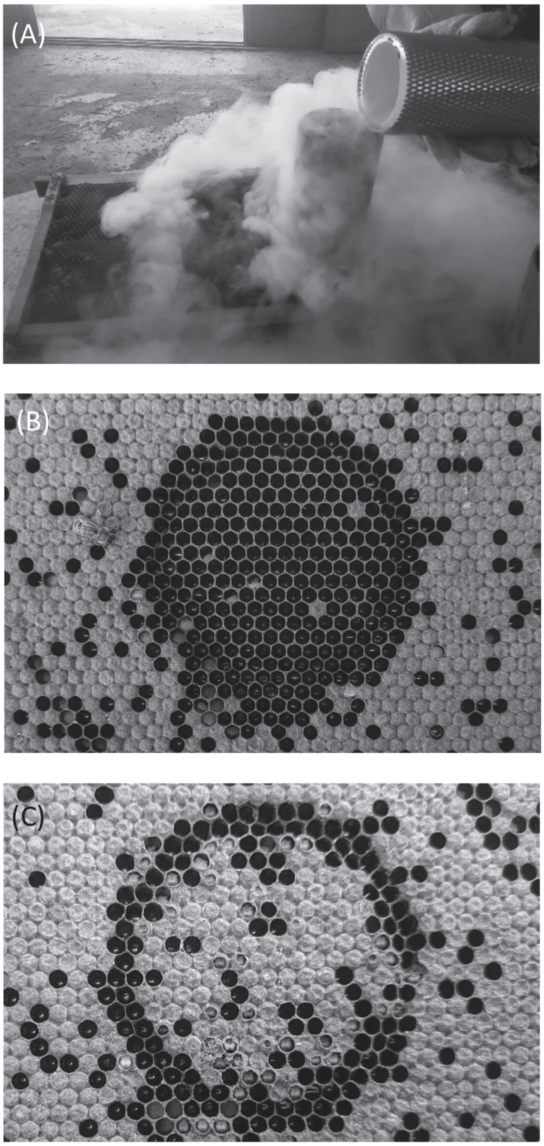

Fig. 1. Evaluation of hygienic behavior in bee colonies with liquid nitrogen. (A) Liquid nitrogen is poured into capped frames. (B) A hygienic bee colony removes dead pupae from the capped frame. (C) A non-hygienic bee colony fails to remove dead pupae from the capped frame.

Comprehensive studies of gene expression in hygienic and control colonies demonstrated that worker bees in hygienic colonies not only have higher gene expression of odorant binding proteins and gustatory receptors but also show increased expression of oxidative phosphorylation pathways in KEGG (Kyoto Encyclopedia of Genes and Genomes). These studies indicate

that signal transduction mechanisms play an important role in inducing hygienic behavior in worker bees (Gempe *et al*. 2016; Morfin *et al*. 2023). Furthermore, worker bees from hygienic colonies have been demonstrated to express higher levels of odorant-binding proteins (OBP3, 16, and 18) on their antennae than those from control colonies. These proteins have an affinity for β-ocimene and oleic acid, which are abundant in 5^{th} instar larvae and prepupae. When mature brood and prepupae were killed by liquid nitrogen, the exudation of β-ocimene and oleic acid induced worker bees to remove the dead brood and prepupae. These studies also found that deformed win virus (DWV, type A and B) titers were higher in control colonies than in hygienic colonies (Mondet *et al*. 2015; McAfee *et al*. 2018). Additionally, Guarna *et al*. (2015) demonstrated differential expression of *BM-40-SPARC* (secreted protein acidic and rich in cysteine Ca binding), Calcyclin-binding protein, and VAMP (vesicle-associated membrane protein) in descendant colonies. The studies indicated that social immunity behaviors are heritable and that biomarkers could potentially be used as indicators for breeding healthy bee colonies.

BREEDING MEASUREMENTS

In a breeding program aimed at enhancing social immunity in colonies, it is essential to monitor social immunity traits, such as grooming and hygienic behaviors, within a genetic resource colony pool. Additionally, monitoring *Varroa* mite infestation rates and virus titers is crucial for selecting parental colonies. *Varroa* mites can transmit the viruses through feeding on bee bodies, with these viruses being detectable in various bee tissues, including mucous glands, seminal vesicles, ovaries, fat bodies, and the midgut. During flight mating, viruses can be transmitted from semen to queens and subsequently to the eggs, leading to latent infections in the progeny colony (Fievet *et al*. 2006; Francis *et al*. 2013; Damayo *et al*. 2023).

Sex determination of honey bees follows the principle where females are diploid and males are haploid, depending on whether the eggs are fertilized (Heimpel & de Boer 2008). Inbreeding often results in the production of diploid males due to homozygosity of complementary sex determination (CSD) genes (Heimpel & de Boer 2008). However, these diploid males do not develop properly and are removed by worker bees, negatively impacting colony development (Ihle *et al*. 2025). Page & Marks (1982) developed a regression model to predict the emergence rate of diploid drone bees, suggesting that with 25 colonies as a genetic resource pool, the survival rate of the bee brood would drop to 85% after 40 generations. Maintaining genetic diversity in a bee breeding program is crucial to prevent the production of diploid drones caused by inbreeding, which decreases the colony productivity and health. This strategy supports breeding colonies with diverse strains, aiding adaptation to changing environmental conditions and disease pressures (Le Conte & Navajas 2008). Furthermore, maintaining diverse populations of lines in a genetic resource pool can provide valuable traits for breeding programs.

Honey bee queens are polyandrous and mate with several drones during one or more mating flights in drone congregation areas (DCAs) (Baudry *et al*. 1998). DCAs consist of sexually mature drones from various colonies, promoting genetic diversity among worker bees in the progeny colony (Baudry *et al*. 1998). This reproductive behavior of the queen causes colony characteristics to vary across generations and creates challenges in tracking paternal lineages.

In a breeding program against *Varroa* mites, it is recommended to rear drones from socially immune colonies for both natural mating and semen collection for artificial insemination (Seltzer *et al*. 2023). Harbo (1976) conducted a study on artificial insemination in

Western honey bees. Artificial insemination of honey bees requires a diluent to dilute the semen, protect the spermatozoa, maintain osmotic pressure, prevent microbial contamination, supply energy to the spermatozoa, and serve as an insemination medium. Ruttner & Drescher (1976) proposed the Kiev solution, followed by the development of a Tris buffer (Rhodes 2008) and a TES buffer (Hopkins *et al*. 2012). These formulations provide proteins to nourish the spermatozoa and reduce oxidative damage (Hopkins & Herr 2010; Rajamohan *et al*. 2020). Cobey (2007) reviewed studies comparing queens inseminated artificially with those mated naturally and found no significant differences in lifespan, fertility, and other key parameters. Factors such as the genetic background of the colony, nutrition, apiary environment, and virgin queen rearing method may influence egg-laying, colony productivity, and the overall colony strength (Cobey 2007; Hasnat 2018; Lee *et al*. 2019; Dolasevic *et al*. 2020).

We referred to the artificial insemination method of honey bees proposed by Cobey *et al*. (2013) (Fig. 2) and observed that the quantity of spermatozoa affects the efficiency of queens in producing fertilized eggs. Providing (4.5 ± 1.2) × 10^6 spermatozoa resulted in approximately (59.6 ± 10.2%) fertilized eggs, while providing (2.4 ± 0.3) × 10^7 spermatozoa resulted in more than (95.2 ± 1.8%) fertilized eggs (Chen 2023). There was no significant difference in the efficiency of producing fertilized eggs between naturally mated queens and artificially inseminated queens with more than a million of spermatozoa. It is suggested that artificial insemination of honey bees using selected drones may facilitate the purification of honey bee strains, breeding, and genetic research. Harbo (1977) attempted to cryopreserve semen in liquid nitrogen for 48 h, resulting in the inseminated queens laying more drones than workers. Gül *et al*. (2017) cryopreserved honey bee spermatozoa for 2 wk, thawed the sperm using a suspension in glucose solution, ram semen plasma, and bee semen plasma, achieving a fertilized egg rate of 40–47% in inseminated queens. Hopkins *et al*. (2012) studied the rate of fertilized eggs in queens artificially inseminated with cryopreserved spermatozoa, which ranged from 17.9–100%, with an average of 49.5%, and found that the queens' lifespans were about 2 mo. Wegener *et al*. (2014) preserved spermatozoa in liquid nitrogen for 9 mo, and the percentage of fertilized eggs produced by queens in the insemination treatment was 59.4%. Overall, although cryopreserved spermatozoa have lower insemination efficacy compared to fresh sperm, they offer the potential to preserve honey bee genetic resources and provide opportunities for the transportation and exchange of germplasm.

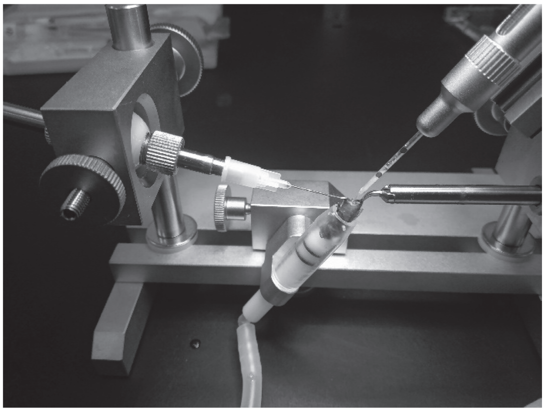

Fig. 2. Instrument used for artificial insemination of a queen bee.

CONCLUSION

In summary, breeding *Varroa* mite-resistant bee strains involves steps as follows: (1) collecting honey bee stocks, (2) assessing the diversity of CSD genes in the stock colonies, (3) evaluating the health status of the stock colonies, (4) assessing the social immune behavioral competencies of the stock colonies, (5) evaluating the expression of social immune behavioral genes, (6) comprehensively assessing the expression of social immune behavioral competencies and genes to select parental

colonies, and (7) evaluating the performance of social immune behavioral traits in offspring colonies. In Taiwan, honey production is an important source of profit for beekeepers. To breed *Varroa* mite-resistant strains of honey bees, we established a honey bee stock consisting of 16 local strains. We surveyed both honey production and hygienic behavior from the bee stock and reared virgin queens from colonies with high honey production and drones from colonies with high hygienic behavior, and then allowed the virgin queens and drones to mate naturally. This procedure was repeated over a period of four years, with one generation reared per year (Fig. 3), resulting in the preliminary establishment of a potentially hygienic bee line (T). The expression level of OBP16 in the T line was significantly higher than that in the control colonies (CK) (Fig. 4B). Although the dead pupa removal rate in the T line was 69.9 ± 10.9%, compared to 43.9–52.3% in the CK (Fig. 4A), the difference was not statistically significant. The resistance traits of honey bees against *Varroa* mites are polygenic and require long-term repeated breeding (Kaskinova *et al*. 2020). It is recommended to conduct comparisons of honey production, *Varroa* mite infestation rates and hygienic behavior to breed *Varroa*-resistant strains of honey bees, contributing to the development of IPM strategies and promoting bee health and sustainable beekeeping.

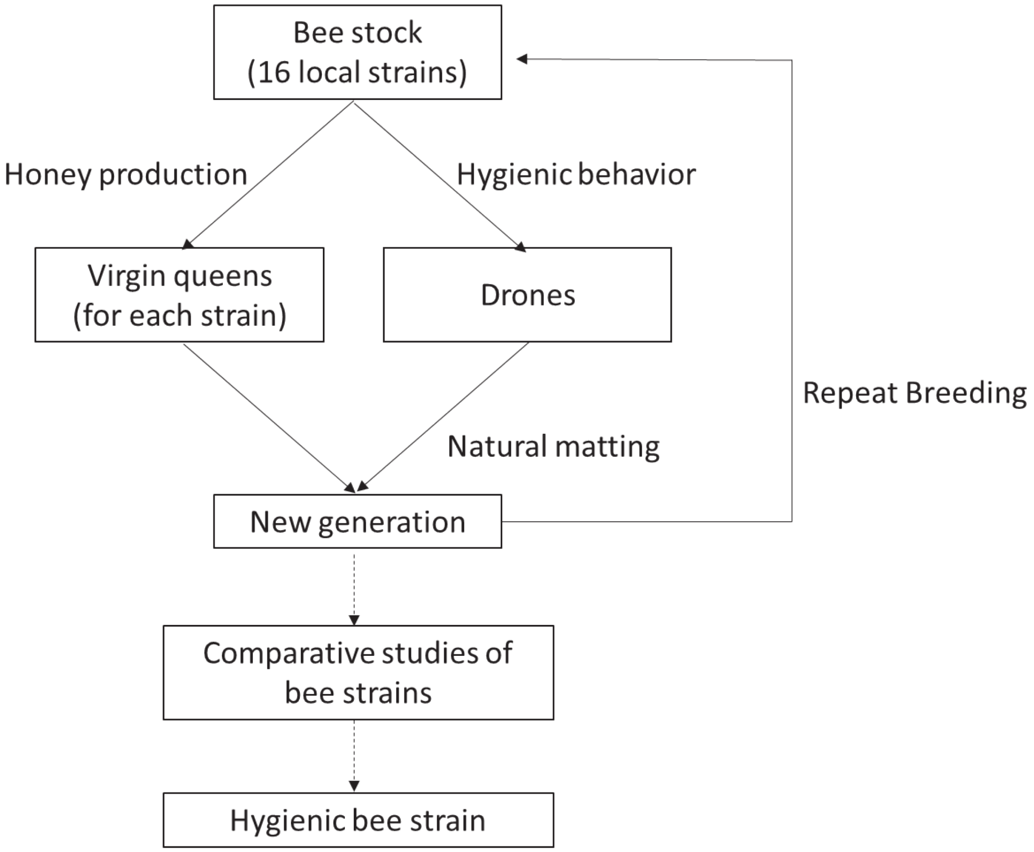

Fig. 3. The process of breeding hygienic strains of Western honey bees.

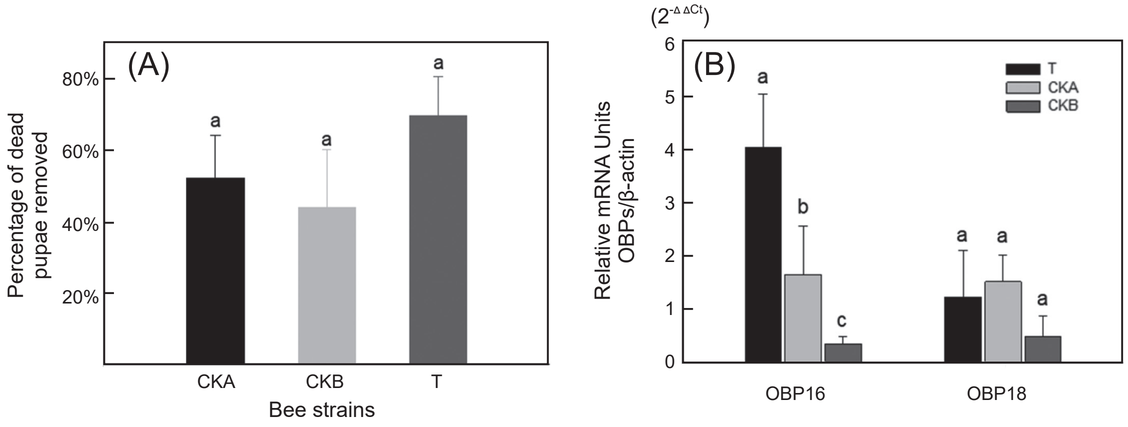

Fig. 4. Preliminary comparative studies of bee strains. (A) Percentage of dead pupae removed: the bee pupae were freeze-killed with liquid nitrogen, and the percentage of dead pupae removed was measured after 24 h. Bar graphs represent the mean (n = 3) ± SD (standard deviation). (B) Relative expression of odorant-binding proteins (OBPs): Different letters on the graph indicate significant differences (Fisher's protected LSD test, P < 0.05). CKA and CKB are control colonies provided by different beekeepers, whereas T represents the hygienic breeding strain.

REFERENCES

Avalos, A., E. Walsh, M. Bixby, A. Card Jr., and W. Card. 2024. A colony health and economic comparison between mite resistant and commercial honey bees (Hymenoptera: Apidae). J. Econ. Entomol. 117:1309–1314. doi:10.1093/jee/toae094

Baudry, E., M. Solignac, L. Garnery, M. Gries, J. Cornuet, and N. Koeniger. 1998. Relatedness among honeybees (*Apis mellifera*) of a drone congregation. Proc. R. Soc. B Biol. Sci. 265:2009–2014. doi:10.1098/rspb.1998.0533

Büchler, R., S. Andonov, K. Bienefeld, C. Costa, F. Hatjina, N. Kezic, ... J. Wilde. 2013. Standard methods for rearing and selection of *Apis mellifera* queens. J. Apic. Res. 52:1–30. doi:10.3896/IBRA.1.52.1.07

Chen, P. H. 2023. Study of artificially inseminated effectiveness in Western honey bee (*Apis mellifera*). MDARES Bull. 12:43–58. (in Chinese with English abstract)

Cobey, S. W. 2007. Comparison studies of instrumentally inseminated and naturally mated honey bee queens and factors affecting their performance. Apidologie 38:390–410. doi:10.1051/apido:2007029

Cobey, S. W., D. R.Tarpy, and J. Woyke. 2013. Standard methods for instrumental insemination of *Apis mellifera* queens. J. Apic. Res. 52:1–18. doi:10.3896/IBRA.1.52.4.09

Cremer, S., S. A. O. Armitage, and P. Schmid-Hempel. 2007. Social immunity. Curr. Biol. 17:R693–R702. doi:10.1016/j.cub.2007.06.008

Currie, R. W. and P. Gatien. 2006. Timing acaricide treatments to prevent *Varroa destructor* (Acari: Varroidae) from causing economic damage to honey bee colonies. Can. Entomol. 138:238–252. doi:10.4039/n05-024

Damayo, J. E., R. C. McKee, G. Buchmann, A. M. Norton, A. Ashe, and E. J. Remnant. 2023. Virus replication in the honey bee parasite, *Varroa destructor*. J. Virol. 97:e01149-23. doi:10.1128/jvi.01149-23

Delaplane, K. S., J. A. Berry, J. A. Skinner, J. P. Parkman, and W. M. Hood. 2005. Integrated pest management against *Varroa destructor* reduces colony mite levels and delays treatment threshold. J. Apic. Res. 44:157–162. doi:10.1080/00218839.2005.11101171

Dietemann, V., F. Nazzi, S. J. Martin, D. L. Anderson, B. Locke, K. S. Delaplane, ... J. D. Ellis. 2013. Standard methods for *Varroa* research. J. Apic. Res. 52:1–54. doi:10.3896/IBRA.1.52.1.09

Dolasevic, S., J. Stevanovic, N. Aleksic, U. Glavinic, N. Deletic, M. Mladenovic, and Z. Stanimirovic. 2020. The effect of diet types on some quality characteristics of artificially reared *Apis mellifera* queens. J. Apic. Res. 59:115–123. doi:10.1080/00218839.2019.1673965

Duay, P., D. De Jong, and W. Engels. 2002. Decreased flight performance and sperm production in drones of the honey bee (*Apis mellifera*) slightly infested by *Varroa destructor* mites during pupal development. Genet. Mol. Res. 1:227–232.

Evans, J. D., K. Aronstein, Y. P. Chen, C. Hetru, J. L. Imler, H. Jiang, ... D. Hultmark. 2006. Immune

pathways and defence mechanisms in honey bees *Apis mellifera*. Insect Mol. Biol. 15:645–656. doi:10.1111/j.1365-2583.2006.00682.x

Fievet, J., D. Tentcheva, L. Gauthier, J. de Miranda, F. Cousserans, M. E. Colin, and M. Bergoin. 2006. Localization of deformed wing virus infection in queen and drone *Apis mellifera* L. Virol. J. 3:16. doi:10.1186/1743-422X-3-16

Food and Agriculture Organization (FAO). 2023. World Food and Agriculture- Statistical Yearbook 2023. Rome, Italy. 384 pp. doi:10.4060/cc8166en

Francis, R. M., S. L. Nielsen, and P. Kryger. 2013. Patterns of viral infection in honey bee queens. J. Gen. Virol. 94:668–676. doi:10.1099/vir.0.047019-0

Gempe, T., S. Stach, K. Bienefeld, M. Otte, and M. Beye. 2016. Behavioral and molecular studies of quantitative differences in hygienic behavior in honeybees. BMC Res. Notes. 9:474. doi:10.1186/s13104-016-2269-y

Gilliam, M., S. Taber III, and G. V. Richardson. 1983. Hygienic behavior of honey bees in relation to chalkbrood disease. Apidologie 14:29–39. doi:10.1051/apido:19830103

González-Cabrera, J., S. Rodríguez-Vargas, T. G. E. Davies, L. M. Field, D. Schmehl, J. D. Ellis, ... M. S. Williamson. 2016. Novel mutations in the voltage-gated sodium channel of pyrethroid-resistant *Varroa destructor* populations from the southeastern USA. PLOS ONE 11:e0155332. doi:10.1371/journal.pone.0155332

Guarna, M. M., A. P. Melathopoulos, E. Huxter, I. Iovinella, R. Parker, N. Stoynov, ... L. J. Foster. 2015. A search for protein biomarkers links olfactory signal transduction to social immunity. BMC Genomics. 16:63. doi:10.1186/s12864-014-1193-6

Gül, A., N. Şahinler, A. G. Onal, B. K. Hopkins, and W. S. Sheppard. 2017. Effects of diluents and plasma on honey bee (*Apis mellifera* L.) drone frozen-thawed semen fertility. Theriogenology 101:109–113. doi:10.1016/j.theriogenology.2017.06.020

Harbo, J. R. 1976. The effect of insemination on the egg-laying behavior of honey bees. Ann. Entomol. Soc. Amer. 69:1036–1038. doi:10.1093/aesa/69.6.1036

Harbo, J. R. 1977. Survival of honey bee spermatozoa in liquid nitrogen1. Ann. Entomol. Soc. Amer. 70:257–258. doi:10.1093/aesa/70.2.257

Harbo, J. R. and J. W. Harris. 2009. Responses to *Varroa* by honey bees with different levels of *Varroa* Sensitive Hygiene. J. Apic. Res. 48:156–161. doi:10.3896/IBRA.1.48.3.02

Harpur, B. A., M. M. Guarna, E. Huxter, H. Higo, K. M. Moon, S. E. Hoover, ... A. Zayed. 2019. Integrative genomics reveals the genetics and evolution of the honey bee's social immune system. Genome Biol. Evol. 11:937–948. doi:10.1093/gbe/evz018

Hasnat, M. A. 2018. Reproductive potential difference of artificially inseminated and naturally mated honey bee queens (*Apis mellifera* L.). Master Thesis. Department of Entomology, Sher-e-Bangla Agricultural University. Dhaka, Bangladesh. 48 pp. doi:10.13140/RG.2.2.16729.77926

Hawkins, G. P. and S. J. Martin. 2021. Elevated recapping behaviour and reduced *Varroa destructor* reproduction in natural *Varroa* resistant *Apis mellifera* honey bees from the UK. Apidologie 52:647–657. doi:10.1007/s13592-021-00852-y

Heimpel, G. E. and J. G. de Boer. 2008. Sex determination in the hymenoptera. Annu. Rev. Entomol. 53:209–230. doi:10.1146/annurev.ento.53.103106.093441

Hopkins, B. K. and C. Herr. 2010. Factors affecting the successful cryopreservation of honey bee (*Apis mellifera*) spermatozoa. Apidologie 41:548–556. doi:10.1051/apido/20010006

Hopkins, B. K., C. Herr, and W. S. Sheppard. 2012. Sequential generations of honey bee (*Apis mellifera*) queens produced using cryopreserved semen. Reprod. Fertil. Dev. 24:1079–1083. doi:10.1071/RD11088

Hung, K. L. J., J. M. Kingston, M. Albrecht, D. A. Holway, and J. R. Kohn. 2018. The worldwide importance of honey bees as pollinators in natural habitats. Proc. R. Soc. Lond. B Biol. Sci. 285:20172140. doi:10.1098/rspb.2017.2140

Ihle, K. E., A. N. Payne, C. G. Elsik, and L. Bilodeau. 2025. Scientific note updating allelic nomenclature standards of the highly diverse complementary sex-determiner locus in honey bees. Apidologie 56:32. doi:10.1007/s13592-025-01161-4

Invernizzi, C., I. Zefferino, E. Santos, L. Sánchez, and Y. Mendoza. 2015. Multilevel assessment of grooming behavior against *Varroa destructor* in Italian and Africanized honey bees. J. Apic. Res. 54:321–327. doi:10.1080/00218839.2016.1159055

Jack, C. J. and J. D. Ellis. 2021. Integrated pest management control of *Varroa destructor* (Acari: Varroidae), the most damaging pest of (*Apis mellifera* L. (Hymenoptera: Apidae)) colonies. J. Insect Sci. 21:6. doi:10.1093/jisesa/ieab058

Johnson, R. M., M. D. Ellis, C. A. Mullin, and M. Frazier. 2010a. Pesticides and honey bee toxicity- USA. Apidologie 41:312–331. doi:10.1051/apido/2010018

Johnson, R. M., Z. Y. Huang, and M. R. Berenbaum. 2010b. Role of detoxification in *Varroa de-*

structor (Acari: Varroidae) tolerance of the miticide tau-fluvalinate. Intl. J. Acarol. 36:1–6. doi:10.1080/01647950903468273

Kaskinova, M. D., L. R. Gaifullina, E. S. Saltykova, A. V. Poskryakov, and A. G. Nikolenko. 2020. Genetic markers for the resistance of honey bee to *Varroa destructor*. Vavilovskii Zhurnal Genet. Selektsii 24:853–860. doi:10.18699/VJ20.683

Khalifa, S. A. M., E. H. Elshafiey, A. A. Shetaia, A. A. A. El-Wahed, A. F. Algethami, S. G. Musharraf, ... H. R. El-Seedi. 2021. Overview of bee pollination and its economic value for crop production. Insects. 12:688. doi:10.3390/insects12080688

Khan, K. A. and H. A. Ghramh. 2021. An investigation of the efficacy of hygienic behavior of various honey bee (*Apis mellifera*) races toward *Varroa destructor* (Acari: Varroidae) mite infestation. J. King Saud. Univ. Sci. 33:101393. doi:10.1016/j.jksus.2021.101393

Le Conte, Y. and M. Navajas. 2008. Climate change: Impact on honey bee populations and diseases. Rev. Sci. Tech. 27:485–510. doi:10.20506/rst.27.2.1819

Lee, K. V., M. Goblirsch, E. McDermott, D. R. Tarpy, and M. Spivak. 2019. Is the brood pattern within a honey bee colony a reliable indicator of queen quality? Insects. 10:12. doi:10.3390/insects10010012

Martin, S. J. 2004. Acaricide (pyrethroid) resistance in *Varroa destructor*. Bee World 85:67–69. doi:10.1080/0005772X.2004.11099632

Masaquiza, D., J. Vargas, N. Ortíz, R. Salazar, L. Curbelo, A. Pérez, and A. Arenal. 2021. Hygienic behavior of *Apis mellifera* and its relationship with *Varroa destructor* infestation and honey production in the central highlands of Ecuador. Insects. 12:966. doi:10.3390/insects12110966

McAfee, A., A. Chapman, I. Iovinella, Y. Gallagher-Kurtzke, T. F. Collins, H. Higo, ... L. J. Foster. 2018. A death pheromone, oleic acid, triggers hygienic behavior in honey bees (*Apis mellifera* L.). Sci. Rep. 8:5719. doi:10.1038/s41598-018-24054-2

Mondet, F., C. Alaux, D. Severac, M. Rohmer, A. R. Mercer, and Y. Le Conte. 2015. Antennae hold a key to *Varroa*-sensitive hygiene behaviour in honey bees. Sci. Rep. 5:10454. doi:10.1038/srep10454

Morfin, N., R. Anguiano-Baez, and E. Guzman-Novoa. 2021. Honey bee (*Apis mellifera*) immunity. Vet. Clin. North Amer. Food Anim. Pract. 37:521–533. doi:10.1016/j.cvfa.2021.06.007

Morfin, N., L. J. Foster, E. Guzman-Novoa, P. Van Westendorp, R. W. Currie, and H. Higo. 2024. *Varroa destructor* economic injury levels and pathogens associated with colony losses in Western Canada. Front. Bee Sci. 2:1355401. doi:10.3389/frbee.2024.1355401

Morfin, N., K. Given, M. Evans, E. Guzman-Novoa, and G. J. Hunt. 2020. Grooming behavior and gene expression of the Indiana "mite-biter" honey bee stock. Apidologie 51:267–275. doi:10.1007/s13592-019-00710-y

Morfin, N., B. A. Harpur, A. De la Mora, and E. Guzman-Novoa. 2023. Breeding honey bees (*Apis mellifera* L.) for low and high *Varroa destructor* population growth: Gene expression of bees performing grooming behavior. Front. Insect Sci. 3:951447. doi:10.3389/finsc.2023.951447

Olate-Olave, V. R., M. Verde, L. Vallejos, L. Perez Raymonda, M. C. Cortese, and M. Doorn. 2021. Bee health and productivity in *Apis mellifera*, a consequence of multiple factors. Vet. Sci. 8:76. doi:10.3390/vetsci8050076

Page, R. E., Jr. and R. W. Marks. 1982. The population genetics of sex determination in honey bees: Random mating in closed populations. Heredity 48:263–270. doi:10.1038/hdy.1982.31

Piou, V., F. Schurr, E. Dubois, and A. Vétillard. 2022. Transmission of deformed wing virus between *Varroa destructor* foundresses, mite offspring and infested honey bees. Parasit. Vectors 15:333. doi:10.1186/s13071-022-05463-9

Porto, R. G., R. F. de Almeida, O. Cruz-Neto, M. Tabarelli, B. F. Viana, C. A. Peres, and A. V. Lopes. 2020. Pollination ecosystem services: A comprehensive review of economic values, research funding and policy actions. Food Secur. 12:1425–1442. doi:10.1007/s12571-020-01043-w

Rajamohan, A., G. D. Robert, B. K. Hopkins, and J. P. Rinehart. 2020. A non-activating diluent to prolong *in vitro* viability of *Apis mellifera* spermatozoa: Effects on cryopreservation and on egg fertilization. Cryobiology 92:124–129. doi:10.1016/j.cryobiol.2019.11.045

Ramsey, S. D., R. Ochoa, G. Bauchan, C. Gulbronson, J. D. Mowery, A. Cohen, ... D. van Engelsdorp. 2019. *Varroa destructor* feeds primarily on honey bee fat body tissue and not hemolymph. Proc. Natl. Acad. Sci. U.S.A. 116:1792–1801. doi:10.1073/pnas.1818371116

Rhodes, J. W. 2008. Semen Production in Drone Honeybees. Rural Industries Research and Development Corporation. Pub. No. 08/130. Wagga Wagga, Australia. 92 pp.

Ricigliano, V. A., S. T. Williams, and R. Oliver. 2022. Effects of different artificial diets on commercial honey bee colony performance, health biomarkers, and

gut microbiota. BMC Vet. Res. 18:52. doi:10.1186/s12917-022-03151-5

Rinderer, T., L. De Guzman, and H. A. Sylvester. 2004. Re-examination of the accuracy of a detergent solution for *Varroa* mite detection. Amer. Bee J. 144:560–562

Ritter, W. 2006. Honey Bee Diseases and Pests: A Practical Guide. FAO Agric. and Food Eng. Tech. Report. Rept. No. 4. Rome, Italy. 42 pp.

Rosenkranz, P., P. Aumeier, and B. Ziegelmann. 2010. Biology and control of *Varroa destructor*. J. Invertebr. Pathol. 103:S96–S119. doi:10.1016/j.jip.2009.07.016

Ruttner, F. and W. Drescher. 1976. The Instrumental Insemination of the Queen Bee. 2nd improved ed. APIMONDIA, International Beekeeping Technology and Economy Institute. Bucharest, Romania. 122 pp.

Seltzer, R., Y. Kamer, P. Kahanov, A. Splitt, M. Bieńkowska, A. Hefetz, and V. Soroker. 2023. Breeding for hygienic behavior in honey bees (*Apis mellifera*): A strong paternal effect. J. Apic. Res. 62:419–428. doi:10.1080/00218839.2022.2140927

Spivak, M. and G. S. Reuter. 2001. Resistance to American foulbrood disease by honey bee colonies *Apis mellifera* bred for hygienic behavior. Apidologie 32:555–565. doi:10.1051/apido:2001103

Stein, K., D. Coulibaly, K. Stenchly, D. Goetze, S. Porembski, A. Lindner, ... E. K. Linsenmair. 2017. Bee pollination increases yield quantity and quality of cash crops in Burkina Faso, West Africa. Sci. Rep. 7:17691. doi:10.1038/s41598-017-17970-2

Torres, D. J. and N. A. Torres. 2020. Modeling the influence of mites on honey bee populations. Vet. Sci. 7:139. doi:10.3390/vetsci7030139

van Alphen, J. J. M., and B. J. Fernhout. 2020. Natural selection, selective breeding, and the evolution of resistance of honeybees (*Apis mellifera*) against *Varroa*. Zoological Lett. 6:6. doi:10.1186/s40851-020-00158-4

Wegener, J., T. May, G. Kamp, and K. Bienefeld. 2014. A successful new approach to honeybee semen cryopreservation. Cryobiology 69:236–242. doi:10.1016/j.cryobiol.2014.07.011

西方蜜蜂提高抗蜂蟹蟎能力之育種方法

陳本翰 [1,*]　黃子豪 [1]　徐培修 [2]　潘其彥 [3]　吳姿嫺 [4]

摘要

陳本翰、黃子豪、徐培修、潘其彥、吳姿嫺。2025。西方蜜蜂提高抗蜂蟹蟎能力之育種方法。台灣農業研究 74(3):237–248。

西方蜜蜂 (*Apis mellifera*) 是重要的授粉昆蟲，對蜂產業生產蜂產品亦具重大貢獻，蜜蜂的健康對糧食安全與農業盈利至關重要。蜜蜂的健康受到蜂蟹蟎 (*Varroa destructor*) 嚴重的威脅，蜂蟹蟎是一種體外寄生蟎，會傳播蜜蜂的疾病與影響蜜蜂的發育，最終影響農業經營收益。為了控制蜂蟹蟎，蜂農傳統上會在蜂巢中使用殺蟎劑，然而，長期使用殺蟎劑會增加蜂蟹蟎抗藥性與蜂產品藥物殘留的風險。除了化學防治，蜜蜂的社會性免疫行為也能提升對蜂蟹蟎的防治效果，這些行為包括工蜂移除死亡的蜜蜂或蜂蟹蟎，減少病原與害蟎在蜂巢內的孳生，而這些社會性免疫行為具有遺傳性。本篇小型綜述聚焦再討論提升蜜蜂抗蜂蟹蟎能力的育種方法，主要包括：(1) 評估種原蜂群的健康狀況；(2) 綜合性評估蜂群的社會性免疫行為能力與基因表現，以篩選親本；(3) 評估子代蜂群社會性免疫行為表現性狀，預期此育種方法將有助於發展更完善的綜合病蟲害管理 (Integrated Pest Management; IPM) 策略。

關鍵詞：西方蜜蜂、蜂蟹蟎、社會性免疫行為、綜合病蟲害管理。

投稿日期：2025 年 3 月 4 日；接受日期：2025 年 5 月 19 日。

[*] 通訊作者：BanHen@mdares.gov.tw
[1] 農業部苗栗區農業改良場蠶蜂科助理研究員。臺灣 苗栗縣。
[2] 農業部苗栗區農業改良場蠶蜂科副研究員。臺灣 苗栗縣。
[3] 農業部苗栗區農業改良場蠶蜂科技佐。臺灣 苗栗縣。
[4] 農業部苗栗區農業改良場蠶蜂科研究員。臺灣 苗栗縣。

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):249–264 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0004

Feature Article

Temperature-Driven Dynamics of Development and Reproduction in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Insights for Advanced Pest Management

Jeong Joon Ahn[1], Jung-Eun Kim[1], Jong-Hwan Shin[1], and Yu-Bing Huang[2,*]

Abstract

Ahn, J. J., J. E. Kim, J. H. Shin, and Y. B. Huang. 2025. Temperature-driven dynamics of development and reproduction in *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae): Insights for advanced pest management. J. Taiwan Agric. Res. 74(3):249–264.

The oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a highly invasive and adaptable agricultural pest, posing significant challenges across diverse ecological and climatic conditions. This study investigates the influence of temperature on the development, longevity, and fecundity of *B. dorsalis* based on the results of laboratory experiments. We analyzed the data using both linear and nonlinear modeling approaches to better understand the temperature-dependent biological characteristics of *B. dorsalis*. These models identify critical thermal thresholds and optimal temperature ranges for providing insights into key life history traits and population dynamics under changing environmental conditions. By integrating temperature-dependent models, this study highlights their potential to refine pest management strategies, including precise timing for interventions such as trap deployment, chemical applications, and sterile insect techniques (SIT). Moreover, these models provide a valuable framework for assessing the impacts of climate change on *B. dorsalis* phenology and geographic distribution, supporting the development of adaptive and sustainable pest management practices tailored to diverse agroecosystems.

Key words: *Bactrocera dorsalis*, Temperature-dependent traits, Population modeling, Pest management, Climate change.

INTRODUCTION

The oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae), is a highly invasive and polyphagous pest that poses substantial threats to global agriculture. It is known to attack over 500 plant species, including both cultivated and wild plants, highlighting its extensive adaptability and ecological plasticity. Among the 85 species in the *B. dorsalis* complex, the identification and differentiation of this species remain challenging due to its morphological and ecological similarities with closely related species such as *B. papayae*, *B. philippinensis*, and *B. invadens* (Drew & Hancock 1994; Clarke *et al*. 2005; Leblanc *et al*. 2013; San Jose *et al*. 2013; Schutze *et al*. 2015). Its geographic range extends from the Asia-Pacific region to parts of Africa, further solidifying its status as a major agricultural pest (Stephens *et al*. 2007; De Villiers *et al*. 2016; Hassani *et al*. 2016).

The life cycle of *B. dorsalis* involves egg deposition into ripening fruit by adult females, where hatched larvae feed and cause significant economic losses. After feeding, larvae pupate in the soil. This species' development is directly

Received: March 5, 2025; Accepted: May 26, 2025.
* Corresponding author, e-mail: ybhuang@tari.gov.tw
1 Research Fellows, Research Institute of Climate Change, National Institute of Horticultural and Herbal Science, Jeju-Si, Korea, ROK.
2 Associate Research Fellow, Applied Zoology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

influenced by environmental conditions, as it does not exhibit an obligatory diapause stage (Christenson & Foote 1960; Hardy 1973). Its rapid adaptation to new environments, coupled with high reproductive capacity, short generation time, and broad climatic tolerance, enables its successful establishment in diverse regions. Furthermore, human activities such as international trade in fruits and vegetables have facilitated its global spread (Leblanc *et al*. 2013; Sridhar *et al*. 2014; Hassani *et al*. 2016; Dong *et al*. 2022; Deschepper *et al*. 2023).

The invasiveness of *B. dorsalis* has been well-documented in various regions. In Taiwan, it was identified as a significant pest in 1912 (Hendel 1912), causing damage to over 20 economically important crops (Huang & Chi 2014). Similar infestations have been recorded in Japan, where eradication strategies such as methyl-eugenol attractants and insecticides achieved temporary success in the 1980s, though reinvasions remain a challenge (Koyama *et al*. 1984; Ohno *et al*. 2009; Otuka *et al*. 2019). In China, *B. dorsalis* has expanded its range into mid-China, though its spread is limited by overwintering constraints near 30°N latitude (Sridhar *et al*. 2014; Hong *et al*. 2015; Magarey *et al*. 2015; Jaffar *et al*. 2023). This ability to persist across diverse ecological and climatic contexts highlights the challenges of managing this species on a global scale.

Temperature plays a pivotal role in regulating the development, survival, and fecundity of *B. dorsalis*. While adults can forage over long distances- up to 38.6 km in some cases (Huang & Chi 2014)- temperature and host plant availability primarily dictate their establishment. Despite extensive research on stage-specific development, there remains a lack of comprehensive models addressing temperature-dependent development, adult longevity, and reproduction. Temperature-dependent oviposition models (OMs) have become essential tools for predicting egg-laying patterns and seasonal population dynamics across a range of insect pests (Baek *et al*. 2024; Lee *et al*. 2024; Sampaio *et al*. 2024). For *B. dorsalis*, characterized by a distinct pre-oviposition period and prolonged adult lifespan, these models must account for daily temperature fluctuations affecting reproduction and survival. Recent advancements, including two-phase OMs that separately model the pre-oviposition and oviposition periods, have improved prediction accuracy under varying temperature conditions. Such models are invaluable for forecasting pest adaptations and optimizing management strategies, particularly in the context of climate change.

In this study, we aim to discuss two interrelated themes: the temperature-dependent development and the combined effects of temperature on longevity and fecundity in *B. dorsalis*. By integrating these two aspects, this review provides a comprehensive understanding of how temperature influences the life history traits and population dynamics of this pest species. The findings will inform the development of predictive models and facilitate the design of effective pest management strategies under diverse environmental conditions.

EXPERIMENTAL FRAMEWORK AND METHODOLOGIES FOR TEMPERATURE-DRIVEN LIFE CYCLE ANALYSIS OF *B. dorsalis*

Colony establishment and maintenance

Colonies of *B. dorsalis* were provided from the Taiwan Agricultural Research Institute (TARI). These colonies, maintained since 1996, were established from a wild population collected in Wufeng County, Taichung, Taiwan. Rearing followed established protocols (Huang & Chi 2014), Larvae were fed on a nutrient-rich artificial diet and provided with sawdust for pupation following the established rearing protocol. Larvae were reared on an artificial diet composed of 5 g of sodium benzoate (Sigma-Aldrich Co., Milan, Italy), 240 g of granulated sugar (Taiwan Sugar corporation, Tainan, Tai-

wan), 140 g of yeast (Vietnam- Taiwan Sugar Company Limited, T Hoa, Vietnam), 20 mL of HCl (Sigma-Aldrich Co., Wien, Austria), 480 g of wheat grain (purchased from a local store, Taichung, Taiwan), and 1,100 mL of water. The pupae were collected by sieving the sawdust. The pupae were moved to a 30 cm × 30 cm container where the adults emerged. The adults were fed an artificial diet composed of 200 g of Yeast Hydrolysate Enzymatic (MP Biomedicals, LLC., Illkirch-Graffenstaden, France), 40 g of granulated sugar, 10 mL of protein hydrolyzate (Alco Standard corporation, Valley Forge, PA), and 50 mL of water. Adults were then provided with a gelatinous mixture, containing water, 100 g of sugar, and 37.5 g of agar (Fei Kung Agar-Agar Co., Ltd, Tainan, Taiwan). Oviposition was induced using guava juice-coated cups (Samayoa *et al*. 2018). The relative humidity was 50–80%, temperature 25 ± 1℃, and photoperiod 14 : 10 (L : D) for mass rearing of *B. dorsalis*.

Temperature-dependent developmental studies

Development rates of immature stages (egg, larva, and pupa) were assessed across eight constant temperatures (13.0 to 34.8℃). Newly deposited *B. dorsalis* eggs (< 3 h old) were collected from the cup by using distilled water. About 100 eggs were inoculated on 9 pieces of filter paper (1 cm × 1 cm), which were placed above a thin layer of artificial diet medium in Petri dishes (90 mm × 15 mm, Alpha Plus Scientific Corp., Taoyuan, Taiwan,). More than 1,000 eggs were placed in environmental chambers (Model A 414931206, Yuh Chuen Chiou Industry Co., Ltd., Kaohsiung, Taiwan) set at eight constant temperatures. Larvae and pupae were reared individually in a controlled environment, with daily observations to determine stage-specific developmental durations. Temperature and humidity (50–80%) in environmental chambers were rigorously maintained, with data used to calculate stage-specific developmental thresholds and rates.

Adult longevity and reproductive performance

One newly emerged (< 12 h) virgin female and two males were placed into a cage (10 cm × 15 cm × 10 cm) with a supply of the artificial diet and a 10% sugar- containing gel. A female with two males produces significantly more eggs than females with only one male (Huang & Chi 2014). Eighteen or twenty cages were treated at each temperature, but a few cages were excluded from the analysis when the female escaped from a cage during egg examination. A perforated plastic cup (diameter and height both 4 cm) with a cotton ball soaked in guava juice placed inside the cup was provided for the oviposition site. The number of eggs laid per female in the cup was recorded daily. The adult oviposition period (AOP) of each female was determined by the day from which the female first laid a significant number (> 5) of eggs consistently, as many females did not lay eggs consistently at 13.5℃, 16.7℃, and 34.9℃. Adult pre-oviposition period (APOP) was defined from adult emergence to AOP, and total pre-oviposition period (TPOP) was obtained by summing the APOP and immature development period examined in the previous study (Samayoa *et al*. 2018). Longevity of males and females was monitored under each temperature conditions to evaluate survival dynamics.

Modeling developmental and reproductive dynamics

Temperature-dependent development rates for all immature stages were analyzed using linear and nonlinear models (Campbell *et al*. 1974; Lobry *et al*. 1991; Rosso *et al*. 1993; Shi *et al*. 2011; Ratkowsky & Reddy 2017). Linear regression was employed to estimate the lower developmental threshold and thermal constant, while nonlinear models such as the Lobry-Rosso-Flandrois (LRF) and performance models captured the thermal responses more comprehensively.

For adults, longevity was modeled using a temperature-dependent survival function, where age-specific survival rates were normalized to physiological age (Curry & Feld-

man 1987). A modified Weibull distribution was used to describe survival probabilities, providing a flexible framework for predicting age-specific survival under varying temperatures. Fecundity patterns were modeled using the two-phase oviposition model, which integrates pre-oviposition and oviposition phases to describe reproductive dynamics comprehensively (Choi *et al*. 2020). The pre-oviposition phase accounts for reproductive maturation, while the oviposition phase predicts daily egg production based on age-specific cumulative oviposition rates. Fig. 1 illustrates the structure and simulation process of the two-phase oviposition model, highlighting its separation into two distinct phases and the integration of physiological age-based predictions for fecundity and survival.

Temperature-dependent developmental models enabled the prediction of adult emergence patterns under fluctuating thermal conditions, providing a foundational framework for simulating population dynamics. The longevity and fecundity of adult *B. dorsalis* were incorporated into the simulation using age- and temperature-dependent models. Longevity was modeled using a Weibull function, normalized to physiological age, to describe survival

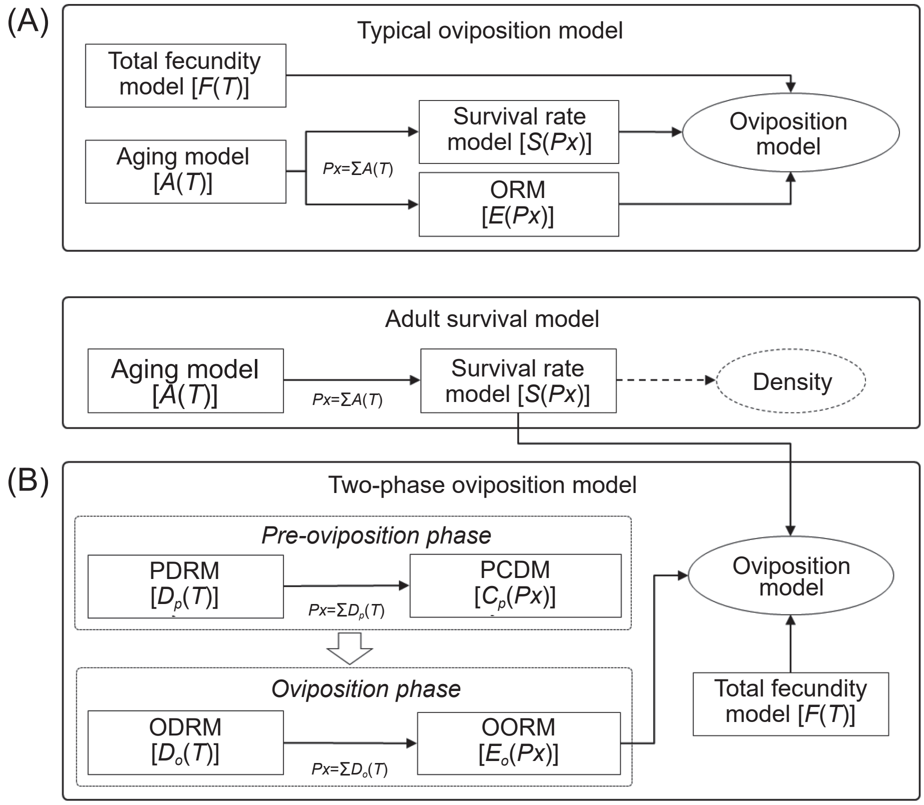

Fig. 1. Illustration of the model structure and simulation process for (A) the current oviposition model (OM) and (B) two-phase OM. (A) The current OM consists of three components; a fecundity model, a female survival rate model, and a cumulative oviposition rate model (ORM), all based on the physiological age (*Px*) derived from the female aging model. (B) The two-phase OM separates the process into two phases. The pre-oviposition phase applies to newly emerged females, where the completion of pre-oviposition is predicted using the pre-oviposition complete distribution model (PCDM) based on the physiological age derived from the pre-oviposition development rate model (PDRM). In the oviposition phase, the daily oviposition is predicted using an age-specific cumulative oviposition rate model (OORM), derived from the oviposition development rate model (ODRM). The daily egg production is calculated as the product of the daily egg proportion, fecundity model, and survival model.

probabilities under varying thermal conditions (Weibull 1951; Pinder *et al*. 1978). For fecundity, the two-phase oviposition model was employed to account for the pre-oviposition and oviposition phases. The pre-oviposition phase used a development rate function to predict the time required for reproductive maturation, while the oviposition phase employed a cumulative Weibull function to describe age-specific oviposition rates. Total fecundity per female was modeled using Brière's equation (Brière *et al*. 1999). These models allowed for precise simulation of daily egg production and reproductive contributions across different temperature regimes.

THERMAL EFFECTS ON THE LIFE CYCLE AND POPULATION DYNAMICS OF *B. dorsalis*

Developmental rates and thermal thresholds

Development occurred at temperatures ranging from 14.4 to 34.8℃. However, failure to progress beyond the egg and larval stages at 13℃ and 14.4℃, respectively, highlights critical lower thermal thresholds for population establishment (Tables 1 and 2). These findings suggest limited survivability in cooler climates. For statistical comparisons among temperature treatments, one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) tests ($P < 0.05$) were performed. Prior to these analyses, the assumptions of normality and homogeneity of variance were evaluated using the Shapiro-Wilk's test. Several traits- including developmental durations (egg, larva, pupa, and total immature)- exhibited significant departures from normality. These deviations are biologically interpretable, as holometabolous insects such as *B. dorsalis* often

Table 1. Development time, median, and hatchability of *Bactrocera dorsalis* eggs across various temperatures.

Temperature (℃)	No.	Development time (d) Mean ± *SE*[z]	Median	Hatchability (%)
13.0	2,500	-[y]	-	0.0
14.4	2,500	7.6 ± 0.04 a	7.1	13.7
16.2	1,930	5.2 ± 0.04 b	4.9	5.9
19.5	2,000	3.0 ± 0.02 c	2.7	42.9
23.8	1,140	2.2 ± 0.02 d	1.8	24.4
27.7	2,300	1.9 ± 0.02 e	1.6	12.3
31.8	1,500	1.5 ± 0.00 f	1.3	27.6
34.8	1,230	2.0 ± 0.02 e	1.8	33.5

[z] Means followed by the same letters in a column are not significantly different ($P < 0.05$, Tukey studentized range test).
[y] Eggs did not hatch at temperature tested.

Table 2. Development time and survival of *Bactrocera dorsalis* larvae and pupae across temperature gradients.

Temperature (℃)	No. examined	Development time (Mean ± *SE* (Median), d)[z]: Larva	Pupa	Larva to adult emergence	Survival (%): Pupation[x]	Adult emergence[w]
14.4	200	34.10 ± 1.09 a (34.5)	-[y]	-	9.5	0.0
16.2	200	19.80 ± 0.84 b (18.9)	29.60 ± 0.75 a (29.0)	53.10 ± 0.60 a (52.5)	69.5	60.0
19.5	200	12.70 ± 0.92 d (11.7)	17.30 ± 0.89 b (16.9)	32.90 ± 0.79 b (32.3)	83.5	75.5
23.8	200	9.30 ± 0.94 e (8.5)	11.20 ± 0.92 c (10.7)	22.20 ± 0.86 c (21.4)	86.5	75.0
27.7	200	8.30 ± 0.92 e (7.5)	9.20 ± 0.92 d (8.4)	18.60 ± 0.85 d (17.5)	65.5	54.0
31.8	200	8.10 ± 0.88 e (7.3)	7.80 ± 0.87 e (7.3)	16.90 ± 0.76 d (16.0)	76.0	42.0
34.8	200	15.30 ± 2.02 c (14.0)	7.00 ± 0.00 e (7.0)	23.70 ± 0.00 c (22.8)	2.0	0.1

[z] Means followed by the same letters in a column are not significantly different, as determined by honestly significant difference (HSD) test at $P = 0.05$.
[y] No pupa developed to the adult stage.
[x] The percent survivorship from larva to pupal stage.
[w] The percent survivorship from larva to adult emergence.

display synchronized developmental transitions under constant environmental conditions. This biological characteristic results in clustered distributions of developmental durations, which inherently deviate from the normal distribution assumption. To address these violations, we applied non-parametric Kruskal-Wallis tests as a robust alternative. The results confirmed significant temperature effects across all measured traits and were largely consistent with the parametric analyses. The convergence of these findings reinforces the robustness and reliability of our interpretation regarding the temperature-dependent developmental responses in *B. dorsalis*. Between 14.4℃ and 31.8℃, development times for all life stages decreased significantly with increasing temperature: egg ($F_{6,\ 2693}$ = 7,986.4, $P < 0.0001$), larval ($F_{6,\ 603}$ = 997.4, $P < 0.0001$), pupal ($F_{5,\ 561}$ = 3,686.1, $P < 0.0001$), and larval to adult stages ($F_{5,\ 591}$ = 4,582.0, $P < 0.0001$). Development duration decreased significantly between 14.4℃ and 31.8℃, with eggs requiring 7.6 to 1.5 d and larval-to-adult stages requiring 53.1 to 16.9 d. Temperature is a key determinant of the development dynamics of *B. dorsalis*, with each life stage exhibiting distinct responses to thermal conditions. Understanding these dynamics is crucial for predicting population trends and implementing effective pest management strategies (Fig. 2). At temperatures exceeding 31.8℃, especially 34.8℃, survival rates declined sharply, indicating a critical upper thermal limit that could severely impact population persistence and geographic distribution. Lower developmental threshold (LDT) ranged from 9.4 to 10.3℃ across life stages, with thermal requirements spanning 33.2–325.5 degree-days (DD) (Table 3).

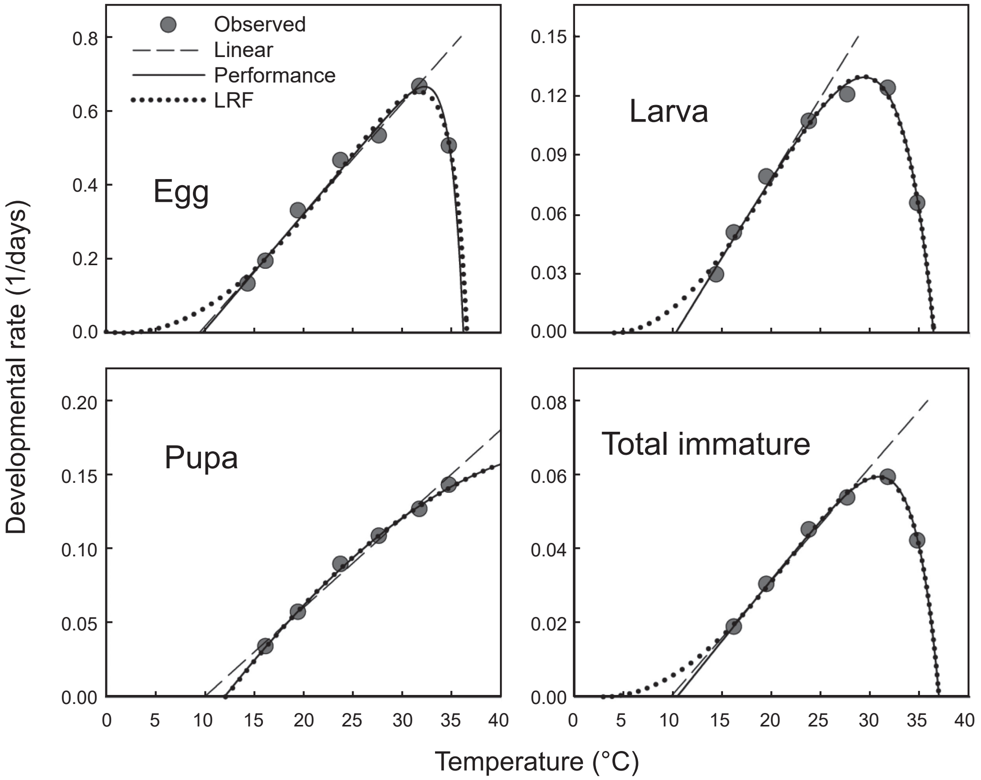

Fig. 2. Comparison of linear and nonlinear models (performance and LRF) fitted to the observed developmental rates (day^{-1}) across life stages (egg, larva, pupa, and total immature) of *Bactrocera dorsalis*. Red points represent observed data, and fitted curves highlight temperature-dependent trends for each stage.

Table 3. Developmental thresholds and thermal requirement for different life stages of *Bactrocera dorsalis*.

Stage^z	Equation	r^2	Lower development threshold (℃)	Thermal constant (degree-day; DD)
Egg	$0.0301\ X - 0.2837$	0.988	9.4	33.2
Larva	$0.0081\ X - 0.0836$	0.987	10.3	123.1
Pupa	$0.0074\ X - 0.0852$	0.986	10.0	166.5
Egg to pupa	$0.0033\ X - 0.0344$	0.990	9.8	325.5

^{z} Egg: $F_{1,4} = 327.05$, $P < 0.0001$; Larva: $F_{1,2} = 143.76$, $P = 0.0069$; Pupa: $F_{1,2} = 216.60$, $P = 0.0006$; Egg to pupa: $F_{1,2} = 205.12$, $P = 0.0048$.

To evaluate the temperature-dependent development of *B. dorsalis*, we compared three candidate models: a linear regression model, Shi's two-phase performance model, and a logistic rate function. These models were applied to four developmental stages- egg, larva, pupa, and total immature- and their performance was evaluated using Akaike information criterion (AIC and AICc), Bayesian information criterion (BIC), and root mean square error (RMSE). Among the tested models, the performance model proposed by Shih consistently provided the best fit across most stages, particularly for total immature development, as indicated by its lowest AIC, BIC, and RMSE values. To further evaluate the predictive robustness of each model, we performed 10-fold cross-validation using the full dataset. The performance model demonstrated strong and consistent predictive accuracy across all developmental stages, either outperforming or equaling the linear and LRF models. Although the linear model occasionally yielded lower RMSE values, its inability to represent biologically meaningful nonlinearities- particularly near thermal limits- limited its ecological applicability. Taken together, the performance model offers the most balanced trade-off between statistical accuracy and biological interpretability under changing temperature conditions. In addition to model performance evaluation, the LRF model offered valuable insights into optimal developmental temperatures, estimating 31.7℃ for eggs, 29.3℃ for larvae, and 30.7℃ for egg-to-adult emergence. These estimates help delineate the thermal tolerance ranges of *B. dorsalis* and inform assessments of its potential ecological adaptability. The completion of developmental stages was further characterized using a two-parameter Weibull distribution, which effectively described the cumulative proportion of development across temperatures (Fig. 3). Simulations based on these models also visualized temperature-dependent adult emergence patterns, showing that 50% cumulative emergence occurred by day 18 under optimal conditions (29–32℃) (Fig. 4). These findings underscore the critical role of temperature in shaping the developmental dynamics and population phenology of *B. dorsalis*.

The longevity and fecundity of *B. dorsalis* adults were significantly influenced by temperature, with variations observed in lifespan, fecundity, and reproductive timing across different thermal conditions (Table 4). To further investigate these effects, we evaluated female and male longevity, fecundity, as well as the preoviposition (APOP) and oviposition (AOP) periods. Normality of the data was assessed using the Shapiro-Wilk test. While adult longevity and oviposition period generally satisfied the assumption, fecundity and preoviposition period deviated from normality in several temperature treatments. Given these deviations, particularly for fecundity and APOP, we applied non-parametric Kruskal-Wallis tests to examine temperature effects. All four traits showed significant variation across temperature treatments. Female longevity decreased from 116.8 d at 18.8℃ to 22.4 d at 34.9℃, while male longevity followed a similar trend, declining from 113.0 d at 13.5℃ to 30.9 d at 34.9℃. These trends highlight the temperature sensitivity of adult survival and its potential effects on population persistence

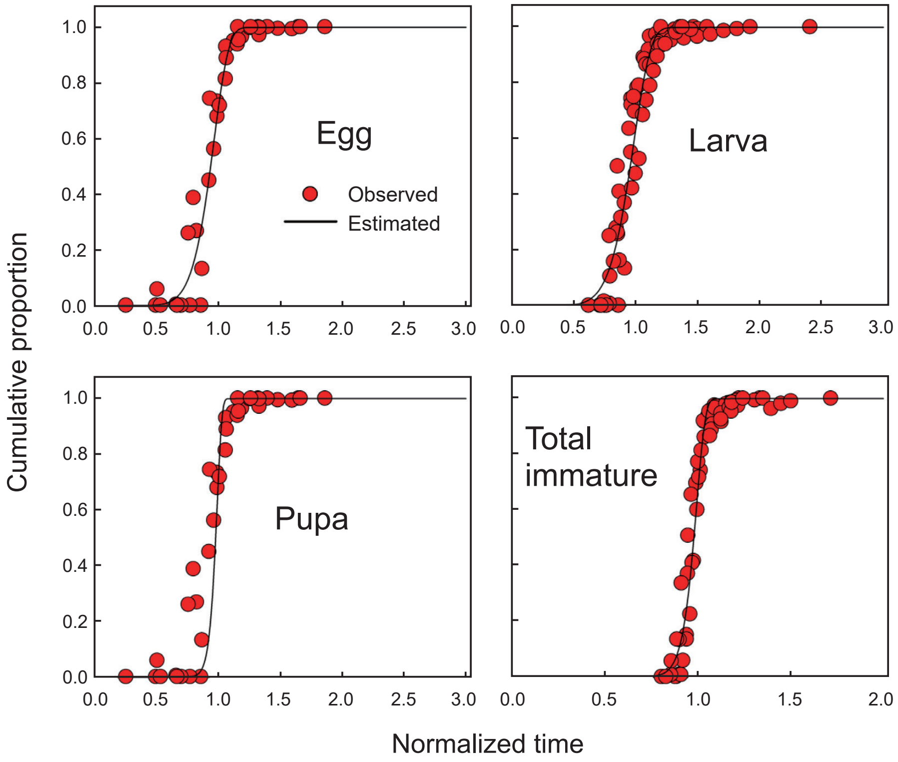

Fig. 3. Cumulative proportions of development completion for each stage of *Bactrocera dorsalis* (egg, larva, pupa, and total immature) as a function of normalized time (developmental time/mean developmental time). Solid lines represent fitted curves based on the two-parameter Weibull function, while red dots indicate observed data.

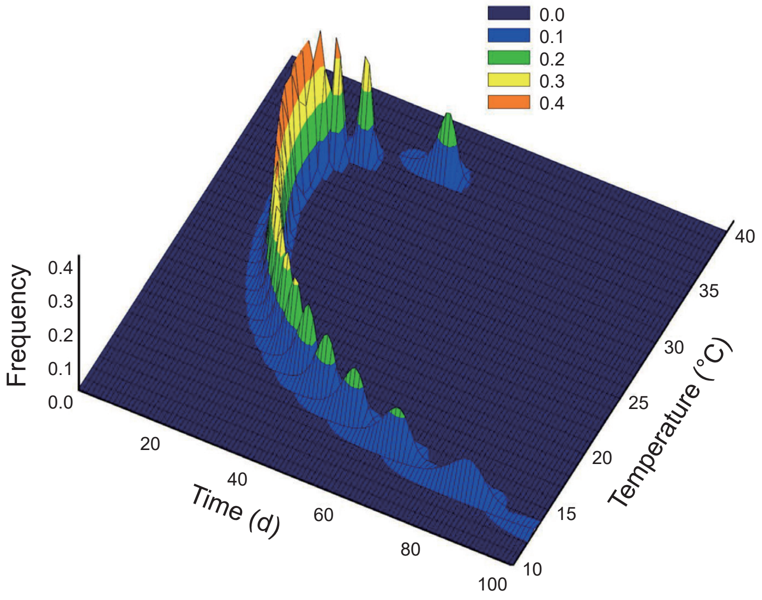

Fig. 4. Simulated adult emergence patterns of *Bactrocera dorsalis* under temperature-dependent conditions based on the performance model.

Table 4. Longevity (d) and fecundity of *Bactrocera dorsalis* adults across various constant temperatures.

	Female			Male	
Temperature (℃)	*n*	Longevity (Mean ± *SE*[z])	Fecundity (Mean ± *SE*)	*n*	Longevity (Mean ± *SE*)
13.5	17	94.10 ± 10.72 (87.0) abc[y]	-[x]	24	113.00 ± 5.68 (122.3) a
16.7	20	53.50 ± 4.46 (54.5) cde	63.0 ± 22.5 c	21	51.40 ± 3.25 (53.8) cde
18.8	18	116.80 ± 7.45 (107.0) ab	922.0 ± 86.7 b	29	79.60 ± 6.35 (76.5) bc
23.5	20	78.50 ± 6.40 (71.0) bcd	1,545.0 ± 160.6 a	36	68.10 ± 4.03 (62.0) bcd
28.1	16	43.30 ± 4.33 (39.0) def	1,684.0 ± 131.9 a	30	43.30 ± 2.08 (44.0) def
32.0	17	42.10 ± 4.63 (37.8) def	958.0 ± 86.9 b	33	30.90 ± 1.79 (28.8) efg
34.9	17	22.40 ± 1.81 (21.5) ef	138.0 ± 34.2 c	34	19.00 ± 0.59 (19.0) fg

[z] Standard error.

[y] Means followed by the same letters in a column are not significantly different, as determined by honestly significant difference (HSD) test at $P = 0.05$. Statistical results: female longevity ($F_{6, 118} = 27.69$, $P < 0.0001$), Fecundity ($F_{6, 118} = 55.57$, $P < 0.0001$) and male longevity ($F_{6, 200} = 68.96$, $P < 0.0001$).

[x] No eggs were laid by the female. Numbers in the parentheses represent the median longevity at each temperature.

under fluctuating climatic conditions. Fecundity peaked at 1,684 eggs per female at 28.1℃, suggesting an optimal temperature for reproduction. Beyond this range, fecundity declined sharply, likely due to thermal stress impairing reproductive physiology ($F_{6, 118} = 27.69$, $P < 0.0001$) (Fig. 5).

Reproductive timing metrics, including the APOP, AOP, and TPOP, also displayed strong temperature dependence. APOP decreased from 38.1 d at 16.7℃ to 4.6 d at 32.0℃ but increased slightly at 34.9℃. Conversely, AOP peaked at 103.1 d at 18.8℃ and declined at both higher and lower temperatures (Table 5). The aging rates of adults increased linearly with temperature above 18.8℃, as modeled using a Weibull distribution. Females exhibited higher survival proportions than males across most temperatures (Fig. 6C and 6D). Thermal constants for female and male longevity were estimated as 507.1 DD and 433.4 DD, respectively (Table 6). Collectively, these findings highlight the profound impact of temperature on adult survival and reproductive performance in *B. dorsalis*. The identification of optimal and limiting temperature thresholds contributes to a better understanding of how temperature governs population persistence and reproductive success. Such insights are essential for developing pre-

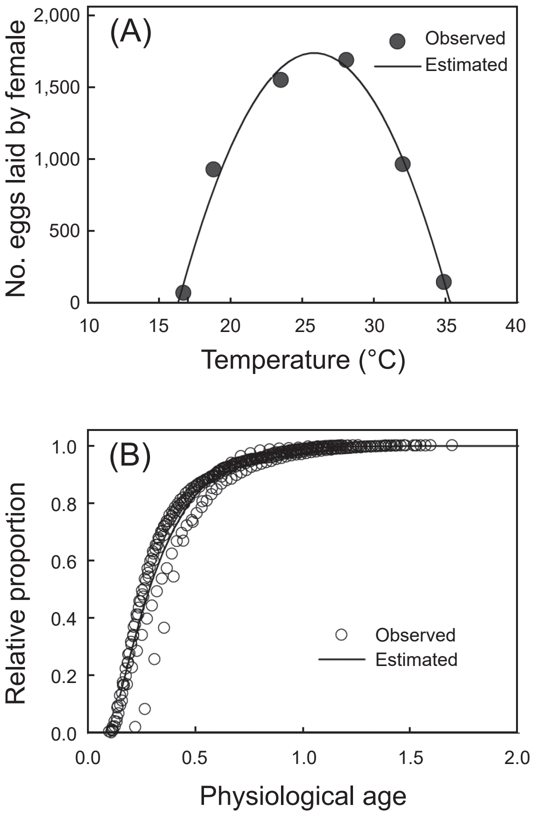

Fig. 5. (A) Fecundity of *Bactrocera dorsalis* females at various temperatures, fitted with a quadratic equation (solid line). (B) Cumulative oviposition probability distribution modeled using a three-parameter Weibull function for females after emergence.

Table 5. Adult pre-oviposition period (APOP) (d), adult oviposition period (AOP), and total pre-oviposition period (TPOP) of *Bactrocera dorsalis* at different temperatures.

Temperature (℃)	No. female oviposited[z]	APOP (Mean ± *SE*[y])	AOP (Mean ± *SE*)	TPOP[x]
16.7	11	38.10 ± 3.06 a[w]	23.60 ± 4.27 c	91.2
18.8	17	16.20 ± 0.75 b	103.10 ± 7.20 a	49.1
23.5	20	9.00 ± 0.21 c	69.50 ± 6.46 b	31.1
28.1	16	5.40 ± 0.58 cd	37.80 ± 4.24 c	24.1
32.0	17	4.60 ± 0.15 d	37.50 ± 4.66 c	21.5
34.9	11	6.20 ± 0.50 cd	17.40 ± 1.55 c	29.9

[z] Female consistently laid a significant number of eggs (> 5).
[y] Standard error.
[x] TPOP represents the sum of APOP and the immature development period, as reported in a previous study (Samayoa *et al*. 2018).
[w] Means with the same letters within a column are not significantly different, as determined by honestly significant difference (HSD) test at $P = 0.05$. Statistical results: APOP ($F_{5, 86} = 130.25$, $P < 0.0001$) and AOP ($F_{5, 86} = 32.09$, $P < 0.0001$).

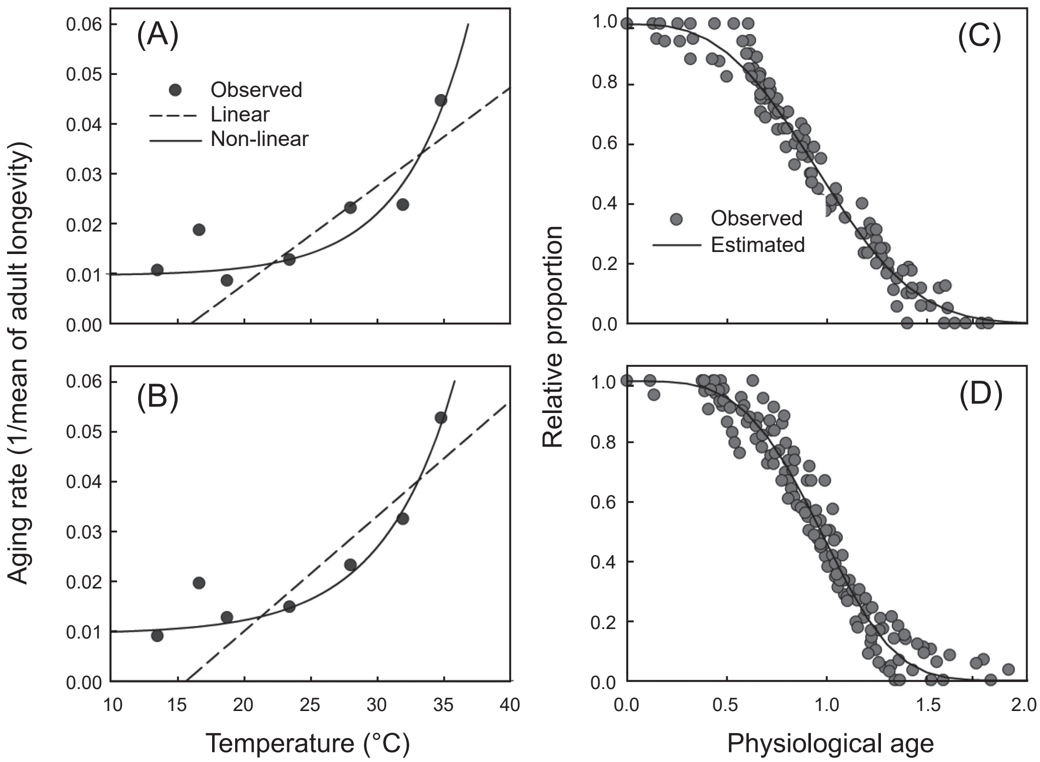

Fig. 6. Aging and survival rates of *Bactrocera dorsalis* adult females and males across various constant temperatures. (A, B) Aging rates (1/mean longevity) for females and males, respectively, fitted with a linear model (dashed line) and a nonlinear model (solid line). (C, D) Survival proportions for females and males, respectively, modeled using a two-parameter Weibull function based on physiological age.

dictive models and adaptive management strategies in the context of climate change.

Fig. 7 provides critical insights into the population dynamics of *B. dorsalis* under varying thermal conditions and offers practical applications for pest management. The comparison between the current oviposition model and the two-phase oviposition model

Table 6. Low developmental threshold (LDT) and thermal constant (TC) for adult longevity, adult pre-oviposition period (APOP), adult oviposition period (AOP), and total pre-oviposition period (TPOP) of *Bactrocera dorsalis*.

Stage	Equation[z]	r^2	LDT (℃)	TC (degree-day; DD)
Male adult longevity	$0.0023X - 0.0363$	0.84	15.7	433.4
Female adult longevity	$0.0020X - 0.0316$	0.83	16.0	507.1
APOP	$0.0126X - 0.1806$	0.99	14.3	79.2
AOP	$0.0014X - 0.0175$	0.89	12.2	696.6
TPOP	$0.0023X - 0.0241$	0.97	10.6	437.4

[z] Linear regression analysis results: Male adult longevity ($F_{1,3} = 15.89$, $P = 0.0283$), Female adult longevity ($F_{1,3} = 15.02$, $P = 0.0304$), APOP ($F_{1,3} = 417.307$, $P = 0.00026$), AOP ($F_{1,2} = 20.5065$, $P = 0.04547$), and TPOP ($F_{1,3} = 96.5145$, $P = 0.00224$).

demonstrates that the latter offers a more nuanced understanding of temperature-driven reproductive performance, particularly under suboptimal or extreme temperatures. By accurately predicting daily egg production across a wide thermal range (10 to 40℃), the two-phase model highlights key thermal thresholds and optimal reproductive temperatures, such as the peak fecundity observed at 28℃. The findings shown in Fig. 7 provide a robust framework for understanding the thermal ecology of *B. dorsalis* and this knowledge can be translated into effective, sustainable, and climate-responsive pest control strategies. By combining these findings from Figs. 4 and 7, pest management strategies can be fine-tuned to effectively suppress populations while minimizing environmental impact. These models represent a significant step toward sustainable, climate-responsive pest management and contribute to broader ecological understanding of temperature-sensitive invasive species.

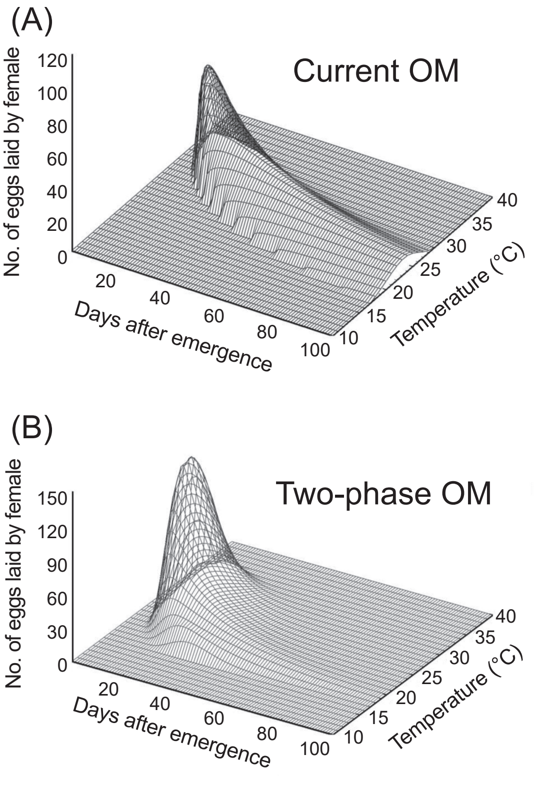

Fig. 7. Predicted daily egg production of *Bactrocera dorsalis* females using (A) the current oviposition model (OM) and (B) the two-phase OM across temperatures ranging from 10 to 40℃ after adult emergence.

PRACTICAL APPLICATION OF TEMPERATURE-BASED MODELS IN PEST MANAGEMENT

The integration of temperature-dependent development and fecundity models offers a robust framework for understanding the population dynamics of *B. dorsalis*. These models enable precise predictions of life stage transitions, reproductive potential, and population fluctuations under changing thermal conditions. Leveraging these insights allows for optimized pest management strategies that minimize economic losses and environmental impacts.

Predicting seasonal population dynamics

One of the primary applications of integrated models is predicting the seasonal activity

patterns of *B. dorsalis*. By incorporating local temperature data into the model, researchers can estimate key developmental milestones, such as larval emergence, pupation, and adult emergence, which are critical timing control measures (Choudhary *et al*. 2025). For instance, in regions with significant temperature fluctuations, models can identify periods of rapid development or delayed emergence, enabling timely interventions. At optimal temperatures (25–30℃), *B. dorsalis* completes its life cycle rapidly, leading to population surges. Predicting these surges allows pest managers to implement preemptive measures, such as deploying traps or applying insecticides during peak activity periods.

Trap deployment and monitoring

Temperature-dependent models guide the strategic placement and timing of placing monitoring traps to control *B. dorsalis* populations. Traps baited with attractants like methyl eugenol effectively capture male flies. Model predictions of adult emergence can optimize trap deployment, ensuring maximum impact (Rincon *et al*. 2024). For instance, traps can be placed in vulnerable orchards before adult emergence during optimal spring temperatures. Early capture of males reduces mating success and population growth. Additionally, predictions of larval development times allow for targeted monitoring of high-risk areas to enhance the precision of pest control efforts. Integrating automated trapping systems with temperature-based models further improves management by predicting life stage transitions such as larval emergence and adult eclosion.

Timing of chemical applications

Chemical control remains a vital component of integrated pest management (IPM) for *B. dorsalis* (Wang *et al*. 2024). Integrated models optimize the timing of insecticide applications by targeting the most vulnerable life stages, such as newly emerged adults or early instar larvae. For instance, if a model predicts a sharp increase in adult emergence within a week due to rising temperatures, insecticides can be applied during this period for maximum efficacy while minimizing non-target effects. Similarly, soil treatments can target pupal stages at their peak density, effectively reducing adult emergence rates.

Supporting sterile insect technique (SIT) programs

Sterile insect technique (SIT) relies on releasing sterile males to reduce wild population fertility (Shelly & Manoukis 2022). Integrated models enhance SIT by predicting peak reproductive periods, allowing for the strategic timing of sterile male releases. In tropical regions with year-round *B. dorsalis* activity, models can identify high reproductive potential periods, ensuring sterile males effectively compete with wild males. Furthermore, models can simulate the impact of different release densities and frequencies, optimizing SIT strategies for varying environmental conditions.

Assessing the impact of climate change

Climate change significantly affects *B. dorsalis* management by altering its geographic range and phenology (Qin *et al*. 2019; Cai *et al*. 2023). Integrated models can simulate how rising temperatures and shifting weather patterns influence population dynamics, supporting proactive management strategies. For instance, in regions not yet invaded by *B. dorsalis* but becoming climatically suitable, models can predict potential invasion risks, enabling early detection and monitoring. Conversely, in areas where temperatures may exceed the species' thermal limits, models can guide adaptive strategies, such as crop diversification or planting schedule adjustments.

Economic and environmental benefits

Applying integrated models reduces reliance on broad-spectrum insecticides, lowering costs and minimizing environmental impacts. By targeting specific life stages and optimizing intervention timing, these models enhance the efficiency and sustainability of pest man-

agement programs (Neta *et al*. 2021). Moreover, by incorporating temperature-dependent parameters, the models ensure that IPM strategies remain adaptable to local environmental conditions and resilient in the face of climate change.

CONCLUSION

The study of temperature-dependent traits, including development, longevity, and fecundity, in *B. dorsalis* provides critical insights into the species' biology and population dynamics. These findings underscore the pivotal role of temperature in shaping key life history traits, such as development rates, reproductive potential, and survival, which collectively influence population growth and geographic distribution.

Integrated modeling approaches, combining empirical data with mechanistic frameworks, are invaluable for understanding these complex interactions. Linear models provide essential metrics, such as LDTs and thermal constants, to estimate phenology and generation times. Meanwhile, nonlinear models, such as LRF and performance models, refine predictions by accounting for the effects of extreme temperatures, offering a more comprehensive understanding of *B. dorsalis* responses to its thermal environment.

The integration of development and fecundity models enhances the ability to simulate population dynamics in detail, paving the way for practical pest management applications. These models enable precise predictions of life stage transitions and reproductive patterns, optimizing interventions such as trap deployment and insecticide application. They also play a crucial role in advanced techniques like the SIT and in adapting pest management strategies that adapt to the challenges of climate change.

As global temperatures rise, adaptive and proactive pest management becomes increasingly critical (Jaffar *et al*. 2023). Integrated models offer a robust framework for predicting how climate change will impact *B. dorsalis* phenology and distribution, ensuring that the continued effectiveness of management programs. Future research should aim to refine these models by incorporating additional environmental factors, such as humidity and photoperiod, and by developing region-specific adaptations to improve their utility.

In conclusion, the comprehensive study and modeling of temperature-dependent traits in *B. dorsalis* mark a significant step forward in sustainable pest management. By merging biological insights with advanced modeling techniques, researchers and practitioners can effectively address the challenges posed by this invasive species, safeguarding both agricultural productivity and ecological stability in a changing climate.

ACKNOWLEDGEMENTS

This research was supported by the research program of Rural Development Administration (PJ017506).

REFERENCES

Baek, S., M. J. Kim, E. Y. Kim, J. K. Jung, and C. G. Park. 2024. Assessment of the occurrence of the second generation of *Mythimna loreyi* Duponchel (Lepidoptera: Noctuidae) using temperature-dependent developmental and oviposition models. PLOS ONE 19:e0303841. doi:10.1371/journal.pone.0303841

Brière, J. F., P. Pracros, A. Y. Le Roux, and J. S. Pierre. 1999. A novel rate model of temperature-dependent development for arthropods. Environ. Entomol. 28:22–29. doi:10.1093/ee/28.1.22

Cai, P., Y. Song, L. Meng, J. Lin, M. Zhao, Q. Wu, ... Q. Ji. 2023. Phenological responses of *Bactrocera dorsalis* (Hendel) to climate warming in China based on long-term historical data. Intl. J. Trop. Insect Sci. 43:881–894. doi:10.1007/s42690-023-00996-7

Campbell, A., B. D. Frazer, N. Gilbert, A. P. Gutierrez, and M. Mackauer. 1974. Temperature requirements of some aphids and their parasites. J. Appl. Ecol. 11:431–438. doi:10.2307/2402197

Choi, K. S., A. C. Samayoa, S. Y. Hwang, Y. B. Huang, and J. J. Ahn. 2020. Thermal effect on the fecundity and longevity of *Bactrocera dorsalis* adults and their improved oviposition model. PLOS ONE 15:e0235910. doi:10.1371/journal.pone.0235910

Choudhary, J. S., S. S. Mali, S. K. Sahu, D. Mukherjee,

B. Das, A. K. Singh, ... B. P. Bhatt. 2025. Predicting abundance and distribution risk of oriental fruit fly, *Bactrocera dorsalis* (Handel) in India based on CMIP6 projections linked with temperature-driven phenology models. J. Agric. Food Res. 19:101613. doi:10.1016/j.jafr.2024.101613

Christenson, L. D. and R. H. Foote. 1960. Biology of fruit flies. Annu. Rev. Entomol. 5:171–192. doi:10.1146/annurev.en.05.010160.001131

Clarke, A. R., K. F. Armstrong, A. E. Carmichael, J. R. Milne, S. Raghu, G. K. Roderick, and D. K. Yeates. 2005. Invasive phytophagous pests arising through a recent tropical evolutionary radiation: The *Bactrocera dorsalis* complex of fruit flies. Annu. Rev. Entomol. 50:293–319. doi:10.1146/annurev.ento.50.071803.130428

Curry, G. L. and R. M. Feldman. 1987. Mathematical Foundations of Population Dynamics. Texas Engineering Experiment Station Monograph Series, No. 3. Texas A&M University Press. College Station, TX. 246 pp.

De Villiers, M., V. Hattingh, D. J. Kriticos, S. Brunel, J. F. Vayssières, A. Sinzogan, ... M. De Meyer. 2016. The potential distribution of *Bactrocera dorsalis*: Considering phenology and irrigation patterns. Bull. Entomol. Res. 106:19–33. doi:10.1017/S0007485315000693

Deschepper, P., S. Vanbergen, Y. Zhang, Z. Li, I. M. Hassani, N. A. Patel, ... H. Delatte. 2023. *Bactrocera dorsalis* in the Indian Ocean: A tale of two invasions. Evol. Appl. 16:48–61. doi:10.1111/eva.13507

Dong, Z., Y. He, Y. Ren, G. Wang, and D. Chu. 2022. Seasonal and year-round distributions of *Bactrocera dorsalis* (Hendel) and its risks to temperature fruits under climate change. Insects. 13:550. doi:10.3390/insects13060550

Drew, R. A. I. and D. L. Hancock. 1994. The *Bactrocera dorsalis* complex of fruit flies (Diptera: Tephritidae: Dacinae) in Asia. Bull. Entomol. Res. Suppl. Ser. 2:1–68. doi:10.1017/S1367426900000278

Hardy, D. E. 1973. The fruit flies (Tephritidae-Diptera) of Thailand and bordering countries. Pacific Insects Monograph. 31:1–353.

Hassani, I. M., L. H. Raveloson-Ravaomanarivo, H. Delatte, F. Chiroleu, A. Alliberti, S. Nouhou, ... P. F. Duyck. 2016. Invasion by *Bactrocera dorsalis* and niche partitioning among tephritid species in Comoros. Bull. Entomol. Res. 106:749–758. doi:10.1017/S0007485316000456

Hendel, F. 1912. H. Sauter's Formosa-Ausbeute. Genus *Dacus*, Fabricius (1805). Suppl. Entomol. 1:13–24.

Hong, S. C., R. Magarey, D. M. Borchert, R. I. Vargas, and S. Souder. 2015. Site-specific temporal and spatial validation of a generic plant pest forecast system with observations of *Bactrocera dorsalis* (oriental fruit fly). NeoBiota. 27:37–67. doi:10.3897/neobiota.27.5177

Huang, Y. B. K. and H. Chi. 2014. Fitness of *Bactrocera dorsalis* (Hendel) on seven host plants and an artificial diet. Türk. Entomol. Derg. 38:401–414.

Jaffar, S., S. A. H. Rizvi, and Y. Lu. 2023. Understanding the invasion, ecological adaptations, and management strategies of *Bactrocera dorsalis* in China: A review. Horticulturae 9:1004. doi:10.3390/horticulturae9091004

Koyama, J., T. Teruya, and K. Tanaka. 1984. Eradication of the oriental fruit fly (Diptera: Tephritidae) from the Okinawa Islands by a male annihilation method. J. Econ. Entomol. 77:468–472. doi:10.1093/jee/77.2.468

Leblanc, L., E. T. Vueti, and A. J. Allwood. 2013. Host plant records for fruit flies (Diptera: Tephritidae: Dacini) in the Pacific Islands: 2. infestation statistics on economic hosts. Proc. Hawaiian Entomol. Soc. 45:83–117.

Lee, H., J. K. Jung, Y. Nam, and S. H. Koh. 2024. Temeprature-dependent oviposition models for *Monochamus saltuarius* (Coleoptera: Cerambycidae). Insects 15:597. doi:10.3390/insects15080597

Lobry, J. R., L. Rosso, and J. P. Flandrois. 1991. A FORTRAN subroutine for the determination of parameter confidence limits in non-linear models. Binary 3:86–93.

Magarey, R. D., D. M. Borchert, G. A. Fowler, and S. C. Hong. 2015. The NCSU/APHIS plant pest forecasting system (NAPPFAST). p.82–96. *in*: Pest Risk Modeling and Mapping for Invasive Alien Species. (Venette, R. C., ed.) Centre for Agriculture and Biosciences International. Wallingford, UK. 240 pp. doi:10.1079/9781780643946.0082

Neta, A., R. Gafni, H. Elias, N. Bar-Shmuel, L. Shaltiel-Harpaz, E. Morin, and S. Morin. 2021. Decision support for pest management: Using field data for optimizing temperature-dependent population dynamics models. Ecol. Model. 440:109402. doi:10.1016/j.ecolmodel.2020.109402

Ohno, S., Y. Tamura, D. Haraguchi, T. Matsuyama, and T. Kohama. 2009. Re-invasions by *Bactrocera dorsalis* complex (Diptera: Tephritidae) occurred after its eradication in Okinawa, Japan, and local differences found in the frequency and temporal patterns of invasions. Appl. Entomol. Zool. 44:643–654. doi:10.1303/aez.2009.643

Otuka, A., M. Matsumura, H. Nakamura, T. Yamaguchi, and Y. Sadoyama. 2019. Possible domestic dispersal

of *Bactrocera dorsalis* during 2015 occurrence on the small islands of south-western Japan. J. Appl. Entomol. 143:556–565. doi:10.1111/jen.12598

Pinder III, J. E., J. G. Wiener, and M. H. Smith. 1978. The Weibull distribution: A new method of summarizing survivorship data. Ecology 59:175–179. doi:10.2307/1936645

Qin, Y., C. Wang, Z. Zhao, X. Pan, and Z. Li. 2019. Climate change impacts on the global potential geographical distribution of the agricultural invasive pest, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae). Climatic Change 155:145–156. doi:10.1007/s10584-019-02460-3

Ratkowsky, D. A. and G. V. P. Reddy. 2017. Empirical model with excellent statistical properties for describing temperature-dependent developmental rates of insects and mites. Ann. Entomol. Soc. Amer. 110:302–309. doi:10.1093/aesa/saw098

Rincon, D. F., E. D. Esch, J. Gutierrez-Illan, M. Tesche, and D. W. Crowder. 2024. Predicting insect population dynamics by linking phenology models and monitoring data. Ecol. Model. 493:110763. doi:10.1016/j.ecolmodel.2024.110763

Rosso, L., J. R. Lobry, and J. P. Flandrois. 1993. An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. J. Theor. Biol. 162:447–463. doi:10.1006/jtbi.1993.1099

Samayoa, A. C., K. S. Choi, Y. S. Wang, S. Y. Hwang, Y. B. Huang, and J. J. Ahn. 2018. Thermal effects on the development of *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) and model validation. Phytoparasitica 46:365–376. doi:10.1007/s12600-018-0674-6

Sampaio, F., M. M. Batista, and C. A. Marchioro. 2024. Temperature-dependent reproduction of *Spodoptera eridania*: Developing an oviposition model for a novel invasive species. Pest Manag. Sci. 80:1118–1125. doi:10.1002/ps.7842

San Jose, M., L. Leblanc, S. M. Geib, and D. Rubinoff. 2013. An evaluation of the species status of *Bactrocera invadens* and the systematics of the *Bactrocera dorsalis* (Diptera: Tephritidae) complex. Ann. Entomol. Soc. Amer. 106:684–694. doi:10.1603/AN13017

Schutze, M. K., N. Aketarawong, W. Amornsak, K. F. Armstrong, A. A. Augustinos, N. Barr, ... A. R. Clarke. 2015. Synonymization of key pest species within the *Bactrocera dorsalis* species complex (Diptera: Tephritidae): Taxonomic changes based on a review of 20 years of integrative morphological, molecular, cytogenetic, behavioural and chemoecological data. Syst. Entomol. 40:456–471. doi:10.1111/syen.12113

Shelly, T. E. and N. C. Manoukis. 2022. Mating competitiveness of *Bactrocera dorsalis* (Diptera: Tephritidae) males from a genetic sexing strain: Effects of overflooding ratio and released females. J. Econ. Entomol. 115:799–807. doi:10.1093/jee/toac027

Shi, P., F. Ge, Y. Sun, and C. Chen. 2011. A simple model for describing the effect of temperature on insect developmental rate. J. Asia-Pac. Entomol. 14:15–20. doi:10.1016/j.aspen.2010.11.008

Sridhar, V., A. Verghese, L. S. Vinesh, M. Jayashankar, and P. D. Kamala Jayanth. 2014. CLIMEX simulated predictions of oriental fruit fly, *Bactrocera dorsalis* (Hendel) (Diptera: Tephritidae) geographical distribution under climate change situations in India. Curr. Sci. 106:1702–1710.

Stephens, A. E. A., D. J. Kriticos, and A. Leriche. 2007. The current and future potential geographical distribution of the oriental fruit fly, *Bactrocera dorsalis* (Diptera: Tephritidae). Bull. Entomol. Res. 97:369–378. doi:10.1017/S0007485307005044

Wang, Y. H., S. L. Wee, S. De Faveri, V. Gagic, S. Hossain, D. F. Cheng, ... Y. Y. Lu. 2024. Advancements in integrated pest management strategies for *Bactrocera dorsalis* in Asia: Current status, insights, and future prospects. Entomol. Gen. 44:1091–1116. doi:10.1127/entomologia/2024/2711

Weibull, W. 1951. A statistical distribution functions of wide applicability. J. Appl. Mech. 18:293–297. doi:10.1115/1.4010337

溫度驅動動態對東方果實蠅 (*Bactrocera dorsalis* (Hendel)) (雙翅目：果實蠅科) 發育與繁殖之探討：蟲害進階管理的見解

安晶駿[1]　金廷恩[1]　辛宗煥[1]　黃毓斌[2,*]

摘要

安晶駿、金廷恩、辛宗煥、黃毓斌。2025。溫度驅動動態對東方果實蠅 (*Bactrocera dorsalis* (Hendel)) (雙翅目：果實蠅科) 發育與繁殖之探討：蟲害進階管理的見解。台灣農業研究 74(3):249–264。

東方果實蠅 (*Bactrocera dorsalis* (Hendel)) 是一種入侵性與環境適應性極強的農業害蟲，在不同的生態與氣候條件都構成防檢疫上重大挑戰。本文將應用線性與非線性模式，論述溫度對東方果實蠅發育、壽命及繁殖力之影響。這些模式確立了其臨界發育閾值與最適發育溫度範圍，藉此瞭解不同環境條件下之生活史關鍵策略與其族群動態。本研究彙整相關溫度依變模式之數值特性，此特性可應用於改善蟲害管理策略之潛力，包括精準規劃防治時機，陷阱設置、化學藥劑施用及昆蟲不孕技術。此外，這些模式可提供生活史關鍵資料作為評估氣候變遷對東方果實蠅之物候學與地理分布的影響，藉此制定不同農業生態系統下適合的韌性策略與永續的害蟲管理。

關鍵詞：東方果實蠅、溫度依變特性、族群模式建置、蟲害管理、氣候變遷。

投稿日期：2025 年 3 月 5 日；接受日期：2025 年 5 月 26 日。
[*] 通訊作者：ybhuang@tari.gov.tw
[1] 韓國氣候變遷暨國家園藝與中草藥科學研究所研究員。韓國 濟州市。
[2] 農業部農業試驗所應用動物組副研究員。臺灣 臺中市。

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):265–273 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0005 **專題論述**

重金屬高富集植物在土壤重金屬污染整治與修復上之應用

陳怡菁[1] 楊純明[2,*]

摘要

陳怡菁、楊純明。2025。重金屬高富集植物在土壤重金屬污染整治與修復上之應用。台灣農業研究 74(3):265–273。

由於長期以來的自然現象與工業及礦業的發展、農業化肥的長期超量使用及工業廢污水的農田灌溉等人為原因，造成全世界的生態系重金屬來源不斷增加與土壤重金屬污染越來越嚴重，臺灣也不例外。土壤重金屬污染牽涉土地利用、國民健康、居家安心及糧食安全等民眾關切的議題，因而普獲各方重視。因此，土壤重金屬污染的整治與修復不僅是重要的環境問題，也是廣被重視的社會與經濟焦點，而且重金屬污染對植物生長造成嚴重威脅，對農糧作物的生產與糧食安全的隱憂當然更是不在話下。據此，近年來開發具有成本效益且高效、環保、低價及易行的植生整治與修復技術來改善重金屬污染的土壤，遂成為環境、地理、土壤及農藝等學術領域關切的研究重點之一。本文整理與介紹一些可利用的重金屬污染整治與修復技術，尤其是相關於植生復育的重金屬高富集植物 (或稱超累積植物，hyperaccumulator plants)，裨益於未來進一步的深入探討及應用。

關鍵詞：重金屬高富集植物、超累積植物、土壤重金屬、污染整治與修復、植生復育。

前言

土壤是地球上最重要的自然資源之一，不僅是支持植物生長所需要的微生物環境，儲存與分解有機物轉換成可被吸收利用的養分以維持生態系統平衡，亦是水資源、碳循環的調節庫及生物多樣性的寶藏。在農業上，土壤也是影響農作物生長、病蟲害、肥培管理、灌溉效率、產量及品質等的重要因素，當受到毒害物質或重金屬污染時，將會產生嚴重的環境問題，更將牽涉到土地利用、國民健康、居家安心及糧食安全等民眾關切的重大議題。然而，除了自然現象之外，工業與礦業的蓬勃發展、農業化肥的大量使用等人為原因，卻造成全世界的生態系重金屬來源不斷增加與土壤重金屬污染越來越嚴重，臺灣也不例外。為求解決重金屬污染對農糧作物生產與糧食安全的隱憂，全球研究莫不聚焦於開發具有成本效益且高效的植物修復技術來整治或修復重金屬污染的土壤。

雖然在低濃度下重金屬是生命過程的重要組成，可協助酶 (酵素) 的製造，參與或催化生物之生理生化過程。然而，當超過特定的臨界值濃度/含量後，這些重金屬對許多生物可能變成毒害元素。植物在接觸重金屬後會經歷氧化逆境，導致細胞損傷，當達一定累積後會擾亂細胞離子的穩定狀態 (cellular ionic homeostasis) (Yadav 2010)。含有高濃度或高含量重金屬的土壤被認為係重金屬污染土壤，將對許多植物/農作物產生毒害甚至於阻礙其生長，因此需要予以整治、修復或改善，以使回歸農業使用。但在進行重金屬污染改善前，應先瞭解重金屬污染物種類、特性、污染範圍、濃度及適用的整治或修復方法，乃至於土壤特性、地下水文地質及當時狀況等，以免反而導致污染擴散而更加難以收拾。

收件日期：2025 年 2 月 8 日；接受日期：2025 年 5 月 8 日。
[*] 通訊作者：cmyang0616@gmail.com
[1] 農業部農業試驗所作物組計畫助理。臺灣 臺中市。
[2] 農業部農業試驗所作物組前研究員兼組長。臺灣 臺中市。

土壤的重金屬污染整治與修復牽涉層面很廣，對各產業的日常運轉與經營發展亦有深遠影響，並且其實務操作非一蹴可幾，作業前需要有充分的瞭解與周詳的規劃。近年來已有許多具有成本效益且高效、環保、低價及易行的植生整治與修復技術來改善重金屬污染的土壤，諸如環境、地理、土壤及農藝等學術領域也都持續積極的從事研究開發。本文整理與介紹一些可供利用的污染整治與修復技術，特別是相關於植生修復的重金屬高富集植物 (或稱超累積植物，hyperaccumulator plants)，裨益於需求者的應用參考與科研人員未來進一步的深入探討。

土壤的重金屬污染整治與修復

土壤或地下水的重金屬污染整治作業有許多修復技術可以採用，惟施行整治與修復之前宜先參考合適的作業指引，依步驟循序進行 (如參閱環境部環境管理署 2024 年 5 月出版之《土壤及地下水重金屬污染整治作業參考指引 (113 年修正版)》)。然而，不同國家地區的情境、環境狀況及經濟條件未必相同，技術種類又形形色色，實施前必須先行瞭解再來評估。通常應用於土壤重金屬污染整治與修復的技術，可概分為農業生態方法、工程方法及生物方法三類型，各有涵蓋內容，每一類型的方法亦皆有多種。如何選用合適的對應方法，端視污染場址的狀態及可採用的技術而異，無可諱言的也會有成本效益的考量。而且在現地生物型整治 (in-situ bioremediation) 實務上，可以將多種技術整合使用，以一種自然程序與環保考量來清除環境中包括重金屬在內的毒害物質。

農藝管理方法

農業生態方法又可分為農田土壤調控與農耕管理措施，前者如土壤 pH 值、水分及氧化還原電位 (Eh) 等項目的調節，後者如改變耕作制度、選用耐抗污染農作物種類與品種及施用合適型態化學肥料等。這些作法主要在於固定土壤中的重金屬、增加土壤對重金屬吸持能力，或減少重金屬被農作物吸收等，但是無法從根本上防治土壤重金屬污染，大多治標不治本。

工程方法

依照《土壤及地下水重金屬污染整治作業參考指引 (113 年修正版)》所述，工程方法係利用物理化學工程措施來整治與修復土壤重金屬污染，包括固化/穩定化、玻璃化法及分離濃縮 (如物理分離、土壤化學淋洗、電熱脫附及電動力法等) 等處理技術在內。簡要的說，固化/穩定化技術係將受污染土壤與固化劑以一定比例混合，改變土壤的理化性質，透過重金屬的吸附或沉澱作用來降低其對生物的效能，而且可緩解重金屬移向土壤深層與地下水，惟僅屬於暫時改變了重金屬在土壤中的存在形態。玻璃化法為將重金屬/廢棄物置於高溫下處理 (常高於 1,000℃)，以電能轉變成熱能將土壤中污染物質破壞分解或固定於成玻璃狀矽酸鹽物質的物理方法，因而降低其移動性。至於物理分離，有污染較不嚴重的耕犁/翻土工法與嚴重污染土壤的排土/換土/客土法 (翻土是將污染重金屬的小區域上層土壤翻至下層，換土是將污染土壤直接取走換入新的未污染土壤，客土稀釋是向污染土壤中加入大量的乾淨土壤予以稀釋)；化學淋洗係使用能促進土壤中非揮發性重金屬遷移或溶解的提取劑 (萃取劑)，使從固相結合的重金屬轉移到液相，再將含有重金屬的液體從土層中抽提出來予以分離與含萃取劑的污水處理；電熱脫附係將揮發性重金屬從土壤顆粒中通入熱蒸汽或熱源加熱等方式加熱土壤，使之從土壤顆粒中分離出來，再收集進行處理；電動力法係將正負電極置於待處理之污染場地施加適當之直流電壓或電流，藉由電極間的電場驅使帶正電荷之離子被吸引到負極、帶負電荷之離子則向正極移動，再將從土壤表面解吸與溶解之聚集於陰極區域的重金屬離子抽提去除。

生物方法

生物方法以植生復育 (或植生修護) (phytoremediation or vegetation remediation) 與微生物處理 (microbial treatment) 為主。植生復育也可稱植物整治，是一項對於環境較為友善、不具侵略性、復育效果明顯及費用較為經濟之技術，又具有安全、操作簡單、可持續性、

環境美學及綠色環保等多項優點而倍受青睞，不破壞環境理化性質、不引起二次污染，係利用植物各種相關新陳代謝反應來達到移除、濃縮及溶解環境重金屬污染物 (Brooks *et al*. 1977; Baker & Brooks 1989; Zhu *et al*. 2014; Guo *et al*. 2019; Ghuge *et al*. 2023)。這些生長於受到重金屬或有機污染物污染土壤、地下水及地面水體之重金屬『高富集 (超累積) 植物』，藉由植株的根部組織提供大量之比表面積，對重金屬具有相當顯著之吸收容量，而且與土壤間具有離子選擇性之傳輸系統，可以自我調整適應於環境中重金屬或污染有機染物之存在。有些植物除了吸收、固定、轉化或累積於根部之外，亦可藉由根部傳導組織將重金屬由土壤中濃縮至植物莖、葉部，或直接降解有機污染物、轉化或礦化成低毒性或無毒性的物質，以及減緩其移動，或促進土壤中微生物將重金屬或有機污染物予以降解或無害化，而達到去除污染物之目的。

經由人工添加或由土壤產生的微生物，來降解或轉移污染物成為低毒性或無毒性的型態，藉以降低或排除污染物濃度，是另一種常用的生物方法。雖然重金屬污染物基本上是不易被微生物降解，但可經由吸附、甲基化、螯合 (chelation) 及價態變化而轉化，因而影響了重金屬的流動性、反應性及生物的利用性。從而可以利用微生物降低重金屬的毒性並防止進一步的污染，如葡萄球菌、芽孢桿菌、假單胞菌、Citrobacteria、Klebsilla 及紅球菌等是常用於微生物復育機制的有機體。透過生物添加物，將微生物與營養物質添加到受污染的場址，或添加營養素與酶來補充及刺激場址的內部微生物活性，都是可行做法。Tiwari *et al*. (2025) 的回顧報告深入探討了高富集植物與其相關微生物群落之間的共生關係，強調根系分泌物與植物免疫如何影響微生物組成，以及這些微生物如何透過各種機制促進重金屬的修復，因此不同的高富集植物可能會以不同的機制進行重金屬修復程序。

上述的植生復育與微生物處理皆為利用某些特定的植物與微生物能夠較快地吸 (除) 去土壤重金屬而達到淨化土壤的目的，相較於其他物理與化學的方法，被歸屬於一種環境友善替代技術。而採用的高富集植物可利用自然生長植物或遺傳工程進行培育，逕行種植於重金屬污染地，微生物則可外添於作物根部附近土壤來改變根部微環境，一方面降低土壤中重金屬的毒性，另方面提高超富集植物對重金屬的吸收、固定，或揮發效率。但是，由於微生物的生物量小，且直接吸收的重金屬量較少，可能也有後續較難實施的清除處理。

重金屬高富集植物

重金屬高富集植物定義為能超量吸收與累積重金屬的植物，乃由 Brooks *et al*. (1977) 提出，又由 Baker & Brooks (1989) 予以整理回顧。一般而言，重金屬高富集植物同時具備 3 個特徵：一是植物地上部 (莖或葉) 重金屬含量是未受到污染或輕度污染的環境下其他植物在同一生長條件下的 10–500 倍 (如鉛的臨界含量為 1,000 mg kg^{-1})，二是植物地上部分的重金屬含量大於根部該種重金屬含量，三是植物的生長沒有出現明顯的毒害症狀 (Baker & Brooks 1989; Liu *et al*. 2007)。生活在重金屬污染程度較高土壤上地上部生物量沒有顯著減少，係超富集植物區別於普通植物的一個重要特徵，可區分出相對於普通植物的超強耐受性 (tolerance) 的表現之一。換言之，高富集植物在重金屬污染的土壤上仍能良好生長，多不會發生毒害現象。部分的普通植物雖然在這種情況下也能生存、生長，但是其地上部分之生物量往往明顯降低，使得植株較矮小，另有部分植物的外觀形態特性還會改變，如葉片大小、花色變色等。Wu (2013) 進一步指出，高富集植物之植株地上部分對重金屬的吸收量比普通植物高 10 倍以上，且不影響正常的生命活動，一般其地上部分的重金屬含量應高於根部重金屬含量：鎳 (Ni)、鉛 (Pb)、鈷 (Co) 等在 1,000 mg kg^{-1} (乾重)，鎘 (Cd) 在 100 mg kg^{-1} (乾重)，錳 (Mn)、鋅 (Zn) 在 10,000 mg kg^{-1} (乾重) 以上。即使在重金屬濃度較低時，高富集植物也有較高積累速率、能同時積累若干種重金屬、生物量大、生長迅速及根系發達等特點。

重金屬高富集植物一般多生長於富含重

金屬的土壤，具有高重金屬耐受性，目前世界上已發現超過 400 多種這類能夠超累積諸如鎘 (Cd)、鉻 (Cr)、銅 (Cu)、鉛 (Pb)、鋅 (Zn)、汞 (Hg)、鎳 (Ni)、鈷 (Co)、錳 (Mn) 及砷 (As) 等重金屬的植物，尤其以分布於熱帶與亞熱帶地區為最多 (Baker & Brooks 1989; Xia *et al*. 2012; Wu 2013; Li *et al*. 2018; Xu *et al*. 2020)。然而在實務操作上，高富集植物仍需要擁有高生物量、生長快速、耐受環境逆境、多種子數量等的特性，以及具有與當地植物競爭的能力，才能夠真正有效應用於植物修復技術。

從自然植物資源來篩選高富集植物的方法，一般係直接在重金屬污染的地方尋找，如礦場、礦區周邊及冶金工業區等。其次，係大量測定一些如花卉、農作物、雜草及牧草等特殊植物體內的重金屬富集程度，惟相對較為耗時費力，也容易忽略一些有修復潛力的植物。Xia *et al*. (2012) 則利用野外採集的土壤種子庫來篩選對重金屬具有高富集特性的植物，透過人為重金屬濃度梯度來驗證其是否具有高富集能力，即經由篩檢添加多種重金屬溶液的土壤中能夠萌發的種子，來選拔出對重金屬有較強耐性的植物種類。這些可以長大又具有高生物量的植物，經測定其植體確實含有高重金屬含量者，就被認定為重金屬高富集植物。此一方法兼顧重金屬耐受性、高富集特性及污染土壤修復能力三項重點，又相對較為省時省力與操作簡易，其關鍵在於土壤種子庫的豐富度、多樣性及代表性。

植物高富集的機制與案例

由於重金屬對植物的毒性與毒害，在自然界中可以觀察到植物也透過迴避 (avoidance) 和耐受機制來對抗重金屬的危害 (Guo *et al*. 2019)，並因此能夠在這些地方定殖，清楚這些機制將可進一步透過植物整治與修復及生物強化途徑來有效的降低或清除土壤重金屬的污染程度 (Viehweger 2014)。從致力於整治與修復重金屬污染的土壤的目的而言，首先需要深入瞭解與植物中重金屬累積及耐受性相關的機制。

近年來許多研究指出，植物根系結構在決定對重金屬逆境或毒性的敏感性或耐受性的過程中扮演著關鍵角色 (Viehweger 2014; Guo *et al*. 2019; Ghuge *et al*. 2023)。某些陸生與水生植物物種被認為是清除重金屬的良好高富集植物，藉由一些轉運蛋白參與重金屬獲取機制，例如 ABC (adenosine triphosphate (ATP)-binding cassette) 轉運蛋白家族、NRAMP 家族 (natural resistance-associated macrophage protein family)、HMA (heavy-metal-associated domain) 及重金屬耐受蛋白等。經由組學工具 (omics tools) 的研究顯示，重金屬的逆境或毒性可以調節高富集植物的多種基因、壓力代謝物或小分子、microRNA 及植物激素等，以促進對高重金屬逆境或毒性的耐受性，進而有效調節其生存代謝途徑 (Ghuge *et al*. 2023)。Yang *et al*. (2020) 從根系對鎘的快速吸收、鎘從根到地上部的有效轉運及植體較強解毒功能 (如植物細胞的區隔化、抗氧化、有機酸生成、滲透物質調節、光合與呼吸作用的維持及氮代謝調節等) 現象，綜述了龍葵高富集鎘的生理及分子機制。Rylott & van der Ent (2025) 評析有關重金屬超積累的分子機制，強調特定轉運蛋白，如 ZIP 家族 (zinc-regulated, iron-regulated transporter-like protein family) 和 NRAMP 家族，在重金屬吸收與轉運中的角色及作用，而且指出抗氧化劑與螯合劑 (如穀胱甘肽與檸檬酸鹽) 有助於減輕重金屬引起的氧化毒害。Xu *et al*. (2020) 研究與分析了 664 種高富集植物之間的系統發育關係 (phylogenetic relationships)，發現近緣物種通常會累積相似的重金屬，顯示重金屬累積特徵具有演化基礎。然而，因為累積特定重金屬的能力各不相同，顯然單憑系統發育仍可能無法預測特定物種之超累積能力。因此，除了各種物理、化學及生物性的重金屬污染土壤整治與修護方法之外，奠基於重金屬耐受植物 (高富集植物) 的形態、生理生化及基因調控機制的綜合應用，污染整治與修復效果將更全面和顯著，也避免了後續二次污染問題。

植物在接觸重金屬後會經歷氧化逆境，導致細胞損傷。此外，植物會累積擾亂細胞離子穩態的金屬離子。而為了最大限度地減少重金屬暴露及其累積的有害影響，某些植物進化出了基於螯合與次細胞區隔化 (subcellular

compartmentalization) 的解毒機制 (Viehweger 2014)。重金屬螯合是許多種植物普遍存在的解毒策略，經由高效率的運輸系統，從敏感位點去除有毒重金屬或將其引導至特定的細胞位置隔離。已知的主要一類螯合劑是植物螯合素 (phytochelatins; PCs)，屬於富含半胱氨酸的胜肽家族 (family of Cys-rich peptides)。PCs 係在植物螯合素合成酶 (phytochelatin synthase; PCS) 催化的轉肽反應中由還原型穀胱甘肽 (reduced glutathione; GSH) 非轉譯合成的 (Yadav 2010)。又在鋅的高富集植物 *Arabidopsis halleri* 案例上，研究發現其根部在過量鋅的環境下，可以分泌能調控植物根圈有效性鋅的螯合劑 (nicotianamine)，據以減緩鋅的吸收速率與降低土壤中鋅的毒性，直接相關於對過量鋅的耐受性 (Tsednee *et al*. 2014)。

以高富集植物整治與修復土壤重金屬污染土地的案例頗多。舉例而言，如對鉻具有高富集特徵的李氏禾 (*Leersia hexandra* Swartz)，被利用於重金屬鉻污染土壤的修復，包括在河川出海口、人工溼地及電鍍工廠廢水污染土地等 (Zhang *et al*. 2011)。由於根系對於鉛的高吸收高富集特性，蓖麻被利用作為大面積鉛污染土壤的植物修復，當植物長到開花期或成熟期時，再將移轉至地上部的植株從污染土壤上移除，實現除去土壤中污染物鉛的目的。如此，既不破壞土壤理化性質與地力，又不引起二次污染，大幅地減少土壤污染運作費用 (Liu *et al*. 2007)。Zhu *et al*. (2014) 精選 3 種礦山鉛污染修復有效植物尼泊爾酸模 (*Rumex nepalensis* Spreng.)、黑麥草 (*Lolium perenne*) 及白三葉草 (*Trifolium repens* Linn.)，藉由混植 (mixed cropping) 提高土壤中重金屬有效態濃度與這些高富集植物生物量，有效地整治與修復礦山重金屬鉛污染。同時混植的方式可增加礦區植被生物多樣性，改善生態功能，發揮長期的正向效果。其他如遏藍菜屬 (*Thlaspi rotundilolium*)、燕麥草 (*Arrhenatherum elatius*) 及愛遏藍菜 (*Thlaspi caerulescens*) 等亦被報導屬於鉛高富集植物，然其等大多生物量小與生長緩慢，大大限制了在修復污染土壤方面的應用 (Liu *et al*. 2007)。

Jing (2013) 則以孔雀草 (*Tagetes patula*)、鳳仙花 (*Impatiens balsamina*) 及腎蕨 (*Nephrolepis cordifolia*) 3 種銅耐性的花卉植物整治與修復銅污染土壤，發現這三種花卉的根系可從污染土壤吸收大量銅並向地上部分轉移，再將採收植株銷售或資源化利用，如此重複若干整治與修復週期的操作清除土壤中的銅污染。Zhong *et al*. (2020) 將多年生草本植物多裂翅果菊 (*Pterocypsela laciniata* (Houtt.) Shih) 幼苗直接移栽或將其種子直接播種於遭受重金屬鎘污染 (5–100 mg kg^{-1}) 的果園土壤中，因為其生物量高且積累量大，在鎘逆境下生長速度較快，可利用於修復果園土壤鎘的污染。Isnard *et al*. (2020) 研究了 New Caledonia 的原生特有種 *Pycnandra acuminata* 對鎳的超累積特性，發現該物種可以耐受高濃度的鎳，其乳膠中的鎳含量高達 257,000 μg g^{-1}，係已知生物體中紀錄的最高值。經由田野調查與栽培試驗，研究發現蜈蚣草 (*Pteris vittata* L.) 對重金屬砷 (As) 具有很強的高富集作用 (Ma *et al*. 2001; Chen *et al*. 2002)。蜈蚣草植體中砷的分布情形與普通植物亦有顯著不同，部位含量高低為葉片 > 葉柄 > 根系，且地上部的生物富集係數 (bioconcentration factors，即生物體內污染物的平衡濃度與其生存環境中該污染物濃度的比值) 隨著土壤含砷量的增加而呈冪函數下降 (Chen *et al*. 2002)。其不僅對砷有很強的耐受能力與富集能力，且生長快、生物量大、地理分布廣及適應性強，對砷污染環境的修復結果甚佳。

在臺灣的實務研究案例上，近年來研究陸續證實十字花科植物具有吸收重金屬污染土壤中鎘與鋅，且累積高濃度鎘與鋅於植體內的現象。Lee *et al*. (2003) 的研究進一步顯示，土壤中重金屬鎘的含量會對根圈土壤中根分泌之雙羧基有機酸的含量造成影響，不論個別或總量皆是。在十字花科根圈中，以主要的雙羧基有機酸物種丙二酸及琥珀酸占有較高的比例 (約 62–100%)；而且土壤中可交換性鎘的含量，明顯導致不同植物根分泌雙羧基有機酸，以受土壤溶液中鎘的驅動而增加分泌與受土壤溶液中的鎘抑制而減低分泌為 2 種主要類型。當土壤溶液中的鎘-雙羧基有機酸複合物含量增加時，將提高植體吸收鎘的含量。Siao

(2011) 調查臺灣高屏溪流域下游地區的河口紅樹林生態系統，發現已成為重金屬的儲存場所，其中蘆葦、海茄苳及馬鞍藤等植體內重金屬含量各異；蘆葦吸收較多的鋅與鎳，平均含量為 72.65 mg kg^{-1} 與 3.31 mg kg^{-1}，海茄苳吸收較多 Cu、Pb 及 Ca，平均含量為 31.31 mg kg^{-1}、3.12 mg kg^{-1} 及 416.84 mg kg^{-1}，馬鞍藤吸收較高的鎘、鉻、鋁及鐵，平均含量為 3.33 mg kg^{-1}、3.99 mg kg^{-1}、5.05 mg kg^{-1} 及 21.12 mg kg^{-1}。其次，沉積物中重金屬生物有效態可被植物體吸收減少，而非生物有效態則被植物根圈吸附固定於沉積物中。而由於這些不同紅樹林植物種類所能吸收累積的重金屬種類不盡相同，因此未來在類似河口或溼地環境區可針對該區受污染種類特性，選擇適當紅樹林植物種類來達到降低重金屬污染的目的。Chen *et al*. (2002) 指出高富集植物的整治與修復效果仍有改進的空間，例如可以透過人為添加物對土壤中的重金屬進行化學活化，以提高重金屬的植物效能；可併行高富集植物與農作物間作，以提高前者的吸收量而使後者產出的農產品不受重金屬危害；將不同重金屬高富集植物進行間作或輪作，以相互促進富集效果；將高富集植物的決定性基因透過基因工程技術轉入生物量較大的植物中，以提高其重金屬去除能力。

Chou *et al*. (2014) 研究指出水稻植株中的砷含量，會受到灌溉用的地下水、土壤中砷的種類與濃度及稻田土壤特性的影響。稻田土壤中存在的亞砷酸鹽會被水稻根部吸收，使得砷保留在根系中，並可轉移到稻株的嫩芽與葉子中，惟因轉移係數較低 (< 0.05)，顯示砷從根部轉移到地上部的比例較低，稻株根組織具有很強的容納砷的能力。Chou *et al*. (2016) 進一步發現，透過灌溉與水管理兩項重要耕作措施，可以控制稻作的生產並調節稻田與稻株中的砷濃度。2013 年一期作的「台農 84 號」與二期作的「台南 11 號」在植株不同部位砷含量隨生育階段與灌溉方式不同而變化，在最大灌溉量的漫灌處理下，稻株在生長過程中體內砷濃度呈現季節性波動；在有氧灌溉與乾濕交替灌溉條件下，稻株中的砷含量低於漫灌下的稻株。顯示不同的灌溉方式可以改變稻田的氧化還原狀況，導致土壤中砷的釋放或吸收，進而影響稻株對砷的吸收。由於稻種之間具有不等積累重金屬能力的差異，未來可以篩檢水稻種子庫找出影響重金屬吸收的基因作為育種上的參考，以確保食用稻米的安全性。

Wang *et al*. (2014) 另在葉用油菜發現能夠在重金屬鎘污染土壤上正常生長的品種，植株地上部與根部含量均較低，或根部重金屬含量雖較高但地上部重金屬含量較低的植物，將之稱為重金屬排異植物 (heavy metal rejecting plants)。此類植物目前尚無公認的篩選標準與方法，且仍有爭議存在，某些研究比照高富集植物的篩選模式，基於可食部位低含量、地上部低富集、體內低轉運及高耐性等標準，以盆栽、小試區或水培等試驗方式，將不同品種可食用部位重金屬含量與國內或國際標準比較，經排序比對而篩選出低富集的重金屬排異品種。但是研究也發現，當土壤重金屬含量超過一定污染閥值時，參試品種可食用部位之重金屬含量皆無法符合食品衛生標準；另外，同一排異品種在不同地區的相近重金屬污染土壤上表現並不相同，出現地域性的差異 (Wang *et al*. 2014)。基此，在篩選重金屬排異品種時，應建議適用的污染地區、污染範圍及污染程度區間，限定其實際可應用的條件。

總體來說，此種排異植物一般在種間與種內皆存在差異，以應用於農業生產上在輕中度污染土壤上相對其他品種累積較低的重金屬為主，當其食用部位符合國家食品衛生標準時，則可以在這些較低污染土壤上進行安全生產。如此一來，既可保障農產品的質量安全，同時又可善用現有的土地資源於農作生產利用 (Wang *et al*. 2014)。Yu *et al*. (2019) 以 2 種具有顯著差異氣候生態區 (29°59′N、102°59′E 與 30°42′N、103°51′E) 之少花龍葵 (又名光果龍葵，*Solanum americanum*) 雜交 F1 植株秸稈施用於輕中程度鎘污染之生菜田區，發現能夠降低生菜植體中鎘的積累及促進生菜的生長，如預期排異植物之表現。基於此一理論，加拿大研究人員於 2004 年選育出硬粒小麥鎘的排異品種 ‘Strongfield’ (Clarke *et al*. 2005)，此品種目標性狀穩定且以商業推廣廣於加拿大種植。美國亦選育出鎘的向日葵排異品種，並成功地推廣栽培 (Tan *et al*. 2023)。

結語

土壤為植物提供了生長所需的水分、營養元素、空氣及地熱等重要因子，亦給予植體機械支撐。惟因隨著工商發展、農業生產及民眾生活等各樣活動所製造產生的污染物長期進入與累積於土壤，形成了土壤污染 (soil pollution)，使得土壤質地逐漸惡化並導致生產力下降，也間接危害人體健康與居住品質。土壤重金屬污染係由污染物中的重金屬或其化合物造成，農地被重金屬污染所生產的糧食如流入食物鏈中，勢將引起嚴重的食安問題及民眾的衛生安全惡果，因此土壤重金屬污染的整治與修復應予重視及優先處理。

雖然土壤的重金屬污染整治作業有許多修復技術可以採用，但是仍須綜合考量國情、社會氛圍、環境狀況及經濟條件，再來評選合適的污染整治與修復技術種類。在農業生態方法、工程方法及生物方法三大類型涵蓋的整治與修復技術當中，以生物方法是已知唯一對環境友善的修復做法，而高富集植物兼顧了重金屬修復、綠色環保及美化環境的多重優點，加上費用低廉、操作簡單安全、較為省時省力、無二次污染顧慮、持續性及全面性等益處，贏得了土壤清道夫美譽而值得採行。本文同時強調，在進行重金屬污染改善前，宜先充分瞭解當地的情境、重金屬污染情形、土壤及水文，再來選用適用的整治與修復方法，在不產生二次污染的前提下，依循自然程序與生態及安全原則予以清除。所回收的重金屬如果可予以資源化的循環再利用，如製成藥品、肥料及純化微量元素等，將可開展出新興產業的循環經濟，共創雙贏。

引用文獻

Baker, A. J. M. and R. R. Brooks. 1989. Terrestrial higher plants which hyperaccumulate metallic elements-A review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126.

Brooks, R. R., J. Lee, R. D. Reeves, and T. Jaffre. 1977. Detection of nickeliferous rocks by analysis of herbarium specimens of indicator plants. J. Geochem. Explor. 7:49–57. doi:10.1016/0375-6742(77)90074-7

Chen, T., C. Wei, Z. Huang, Q. Huang, Q. Lu, and Z. Fan. 2002. Arsenic hyperaccumulator plant Torenia concolor and its arsenic accumulation characteristics. Chin. Sci. Bull. 47:207–210. (in Chinese) doi:10.1360/csb2002-47-3-207

Chou, M. L., J. S. Jean, G. X. Sun, Z. Y. Hseu, C. M. Yang, S. Das, and J. H. Teng. 2014. Distribution and accumulation of arsenic in rice plants grown in arsenic-rich agricultural soil. Agron. J. 106:940–951. doi:10.2134/agronj13.0497

Chou, M. L., J. S. Jean, G. X. Sun, C. M. Yang, Z. Y. Hseu, S. F. Kuo, ... Y. J. Yang. 2016. Irrigation practices on rice crop production in arsenic-rich paddy soil. Crop Sci. 56:422–431. doi:10.2135/cropsci2015.04.0233

Clarke, J. M., T. N. McCaig, R. M. DePauw, R. E Knox, F. R. Clarke, M. R. Fernandez, and N. P. Ames. 2005. Strongfield durum wheat. Can. J. Plant Sci. 85:651–654. doi:10.4141/P04-119

Ghuge, S. A., G. C. Nikalje, U. S. Kadam, P. Suprasanna, and J. C. Hong. 2023. Comprehensive mechanisms of heavy metal toxicity in plants, detoxification, and remediation. J. Hazard. Mater. 450:131039. doi:10.1016/j.jhazmat.2023.131039

Guo, Y., J. Ma, P. Jiang, J. Li, J. Gao, S. Qiao, and Z. Zhao. 2019. The mechanism of plant resistance to heavy metal. IOP Conf. Ser. Earth Environ. Sci. 310:052004. doi:10.1088/1755-1315/310/5/052004

Isnard, S., L. L'Huillier, A. L. D. Paul, J. Munzinger, B. Fogliani, G. Echevarria, ... A. van der Ent. 2020. Novel insights into the hyperaccumulation syndrome in *Pycnandra* (Sapotaceae). Front. Plant Sci. 11:559059. doi:10.3389/fpls.2020.559059

Jing, Y. 2013. Method for Repairing Cooper Contaminated Soil by Using Three Copper Resistance Flower Plants. Pub. No. CN102935446A. Qufu, China. https://patents.google.com/patent/CN102935446A/zh (visit on 1/25/2025) (in Chinese with English abstract)

Lee, C. H., C. Y. Chiu, Y. M. Tzou, P. N. Chiang, and M. K. Wang. 2003. Effects of soil cadmium and zinc heavy metal contaminants on plant root exudates of low molecular organic acids. Soil Environ. 6:9–16. (in Chinese with English abstract) doi:10.30058/SE.200303.0003

Li, J. T., H. K. Gurajala, L. H. Wu, A. van der Ent, R. L. Qiu, A. J. M. Baker, ... W. S. Shu. 2018. Hyperaccumulator plants from China: A synthesis of the current state of knowledge. Environ. Sci. Technol. 52:11980–11994. doi:10.1021/acs.est.8b01060

Liu, X., Y. Gao, B. He, Z. Wang, P. Ning, X. Zeng, ... B. Wen. 2007. Application and Method of Utilizing Enriching Plant in Repairing Lead Polluted Soil.

Pub. No. CN1887457A. Yunnan Institute of Environmental Sciences. Kunming, China. https://patents.google.com/patent/CN1887457A/zh (visit on 1/22/2025) (in Chinese with English abstract)

Ma, L. Q., K. M. Komar, C. Tu, W. Zhang, Y. Cai, and E. D. Kennelley. 2001. A fern that hyperaccumulates arsenic. Nature 409:579. doi:10.1038/35054664

Rylott, E. L. and A. van der Ent. 2025. Harnessing hyperaccumulator plants to recover technology-critical metals: Where are we at? New Phytol. 246:859–866. doi:10.1111/nph.20449

Siao, M. F. 2011. Uptake and accumulation of heavy metals in sediment by mangroves. Master Thesis. Department of Environmental Engineering and Science, Fooyin University. Kaohsiung, Taiwan. 106 pp. (in Chinese with English abstract)

Tan, D., L. Zhang, S. Zhang, and B. Cui. 2023. Physiological response of four widely cultivated sunflower cultivars to cadmium stress. Horticulturae 9:320. doi:10.3390/horticulturae9030320

Tiwari, S., A. Ullah, Y. X. Feng, and X. Z. Yu. 2025. Exploring plant symbiotic microbial dynamics in metal hyperaccumulators for phytoremediation. Intl. Biodeterior. Biodegrad. 196:105941. doi:10.1016/j.ibiod.2024.105941

Tsednee, M., S. C. Yang, D. C. Lee, and K. C. Yeh. 2014. Root-secreted nicotianamine from *Arabidopsis halleri* facilitates zinc hypertolerance by regulating zinc bioavailability. Plant Physiol. 166:839–852. doi:10.1104/pp.114.241224

Viehweger, K. 2014. How plants cope with heavy metals. Bot. Stud. 55:35. doi:10.1186/1999-3110-55-35

Wang, L., Y. Xu, X. Liang, Y. Sun, D. Lin, Y. Sun, and X. Qin. 2014. Method for Screening Cadmium Rejection Varieties of Leaf-Edible Rapes. Pub. No. CN103238439A. Agro-Environmental Protection Institute, Ministry of Agriculture. Tianjin, China. https://patents.google.com/patent/CN103238439A/zh (visit on 1/22/2025) (in Chinese with English abstract)

Wu, J. 2013. Island ecological restoration and environmental protection. China Ocean Press. Beijing, China. 383 pp. (in Chinese)

Xia, H., X. Zhang, Z. Li, and P. Zhuang. 2012. Method for Searching Heavy Metal Hyper Accumulator. Pub. No. CN102160497A. South China Botanical Garden of CAS. Guangzhou, China. https://patents.google.com/patent/CN102160497A/zh (visit on 1/25/2025) (in Chinese with English abstract)

Xu, W., P. Xiang, X. Liu, and L. Q. Ma. 2020. Closely-related species of hyperaccumulating plants and their ability in accumulation of As, Cd, Cu, Mn, Ni, Pb and Zn. Chemosphere 251:126334. doi:10.1016/j.chemosphere.2020.126334

Yadav, S. K. 2010. Heavy metals toxicity in plants: An overview on the role of glutathione and phytochelatins in heavy metal stress tolerance of plants. S. Afr. J. Bot. 76:167–179. doi:10.1016/j.sajb.2009.10.007

Yang, X. Y., H. J. Wang, and H. B. Wang. 2020. Advances in physiological and molecular mechanisms of cadmium hyperaccumulation by *Solanum nigrum* L. Asian J. Ecotoxicol. 15(6):72–81. (in Chinese with English abstract)

Yu, X., Y. Tang, Y. Xie, H. Li, H. Tan, L. Wang, ... M. Liao. 2019. A Method to Reducing Cadmium Accumulation in Lettuce by Applying *Solanum nigrum* Straw. Pub. No. CN109848205A. Sichuan Agricultural University. Sichuan, China. https://patents.google.com/patent/CN109848205A/zh (visit on 1/30/2025) (in Chinese with English abstract)

Zhang, X., J. Liu, and Y. Zhu. 2011. Research on Phytoremediation Technology of Heavy Metal-Contaminated Soil: Study on the Hyperaccumulation Characteristics, Mechanism and Remediation Potential of Chromium by *Leersia hexandra*. China Science Publishing & Media. Beijing, China. 164 pp. (in Chinese)

Zhong, L., X. Li, D. Xia, P. Liu, H. Li, M. Liao, ... H. Xia. 2020. A Kind of Method for Repairing Cadmium-Contaminated Orchard Soil of *Pterocarpus multiflora*. Pub. No. CN108326039B. Sichuan Agricultural University. Sichuan, China. https://patents.google.com/patent/CN108326039B/zh (visit on 1/25/2025) (in Chinese with English abstract)

Zhu, X., Y. Yang, J. Shao, Z. Yang, and H. Yang. 2014. Remediation Method for Heavy Metal Lead Pollution in Mine. Pub. No. CN102847707A. Sichuan Agricultural University. Sichuan, China. https://patents.google.com/patent/CN102847707A/zh (visit on 1/20/2025) (in Chinese with English abstract)

Application of Hyperaccumulator Plants in the Remediation and Restoration of Heavy Metal Polluted Soils

Yi-Jing Chen[1] and Chwen-Ming Yang[2,*]

Abstract

Chen, Y. J. and C. M. Yang. 2025. Application of hyperaccumulator plants in the remediation and restoration of heavy metal polluted soils. J. Taiwan Agric. Res. 74(3):265–273.

Due to long-term natural phenomena and other man-made activities such as the development of industrial and mining enterprises, excessive use of agricultural fertilizers and farmland irrigation with industrial wastewater, the sources of heavy metals in the world's ecosystems have continued to increase and heavy metal pollution in soils has become more and more serious. Taiwan is no exception. Soil heavy metal pollution involves issues of public concern such as land use, national health, home security, and food security, and therefore has received widespread attention from all parties. As such, the remediation and restoration of soil heavy metal pollution is an important environmental concern and a widely valued social and economic focus. Heavy metal pollution poses a serious threat to plant growth and hidden worries to the production of food crops and food security. In recent years, the development of cost-effective, efficient, environmentally friendly, low-priced, and easy vegetation remediation technologies to remediate heavy metal-contaminated soil has become one of the research focuses of academic fields such as environment, geography, soil, and agronomy. This article collates and introduces some available heavy metal pollution remediation and restoration technologies, especially the heavy metal hyperaccumulator plants related to phytoremediation, which will benefit further in-depth research and application in the future.

Key words: Heavy metal hyperaccumulator plants, Hyperaccumulating plants, Soil heavy metals, Pollution remediation and restoration, Phytoremediation.

Received: February 8, 2025; Accepted: May 8, 2025.

* Corresponding author, e-mail: cmyang0616@gmail.com

1 Project Assistant, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

2 Former Research Fellow and Division Director, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):275–289 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0006

Research Article

Survey of Passionfruit Viruses in Okinawa, Japan: Prevalence, Transmission, and Control of Passiflora Latent Virus Using Virus-Free Seedlings

Atsushi Ajitomi[1,*], Akari Saito[2], Shinji Kawano[3], and Ken-taro Sekine[4,*]

Abstract

Ajitomi, A., A. Saito, S. Kawano, and K. T. Sekine. 2025. Survey of passionfruit viruses in Okinawa, Japan: Prevalence, transmission, and control of Passiflora latent virus using virus-free seedlings. J. Taiwan Agric. Res. 74(3):275–289.

In Okinawa Prefecture, passionfruit cultivation during the winter and spring seasons has become problematic due to the emergence of symptoms, e.g., shriveled new leaves and reduced plant vigor. In 2017, a survey of 84 fields was conducted across the region. The symptoms of suspected virus diseases were confirmed, and the outbreaks of 6 specific viruses in Japan were further investigated via reverse transcription-polymerase chain reaction (RT-PCR). The results showed that the top three common symptoms were the curling of adult leaves, leaf surface cracking, and yellowing between leaf veins. Among the 6 viruses tested, East Asian Passiflora distortion virus (EAPDV), East Asian Passiflora virus-AO (EAPV-AO), and Passiflora latent virus (PLV) gave an incidence of 2.4%, 1.2%, and 85.7%, respectively, whereas Broad bean wilt virus-2 (BBWV-2), Cucumber mosaic virus (CMV), and EAPV-IB were not detected. Plants infected with EAPDV and EAPV-AO showed typical symptoms of leaf shriveling and fruit malformation, but those infected with PLV showed several other nonspecific symptoms. Based on the survey results, PLV was identified as the primary pathogen. The virus was isolated from infected passionfruit, causing symptoms in quinoa but not in passionfruit. PLV was also confirmed to be transmitted by insect vectors, e.g., cotton aphids, as well as by juice remaining on pruning shears. Furthermore, as PLV was detected in passionfruit seedlings obtained from a supplier, thus the supply of confirmed PLV-free seedlings was established as a countermeasure in Okinawa starting from 2019. Since then, the occurrence of PLV has dramatically decreased, as revealed in a second field survey conducted in 2022 showing a significantly lower number of PLV-infected fields. Abnormal symptoms of passionfruit and high incidence of PLV infection showed a strong positive correlation in Okinawa.

Key words: Aphid, Pruning shears, RT-PCR, DECS-C, *Carlavirus*.

INTRODUCTION

Passionfruit is a perennial plant belonging to genus *Passiflora*. There are approximately 400 known species in this genus, most of them originating from tropical and subtropical South America. Of these species, only approximately 50–60 are edible. The main passionfruit species cultivated in Japan is *Passiflora edulis* Sims, known as the purple species or native species. Passionfruit is grown in Kagoshima Prefecture, Okinawa Prefecture, and Tokyo, mainly on the Ogasawara

Received: May 9, 2025; Accepted: June 9, 2025.
* Corresponding author, e-mail: ajitomia@pref.okinawa.lg.jp; k-sekine@cs.u-ryukyu.ac.jp
1 Senior Researcher, Okinawa Prefectural Agricultural Research Center, Okinawa, Japan.
2 Graduate Student, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.
3 Research Advisor, Okinawa Prefectural Plant Protection Association, Okinawa, Japan.
4 Associate Professor, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.

Islands (Yonemoto & Kondo 2020). In Okinawa Prefecture, passionfruit cultivation began in the 1960s, and the cultivation area has been expanding each year. In recent years, however, abnormal virus-like symptoms, e.g., the shriveling of new leaves and a declined vigor, have been observed in passionfruit fields in Okinawa Prefecture during the winter/spring season. Passionfruit seedlings are propagated by cuttings, and if an infection is established via sap-sucking aphids or other vectoring insects, the seedlings continue to carry the virus. Therefore, there is a concern that the disease may be spread through propagated seedlings supplied by seed and plant companies.

In Japan, the outbreaks of five major viruses infecting passionfruit have been reported, i.e., Cucumber mosaic virus (CMV) (Yonaha *et al*. 1979), Broad bean wilt virus 2 (BBWV-2) (Yonaha *et al*. 1993; Kobayashi *et al*. 2004), Passiflora latent virus (PLV) (Watanabe *et al*. 1997), East Asian Passiflora virus (EAPV) (Iwai *et al*. 2006), and East Asian Passiflora distortion virus (EAPDV) (Riska *et al*. 2019). In 2020, Nakasato *et al*. (2020) reported the detection of two potyviruses, Uraria mosaic virus and Passiflora foetida virus Y (PfVY), in passionfruit in Japan. Globally, more than 20 viruses have been reported in plants of genus *Passiflora*, and it is possible that additional, yet-undiscovered, viral species exist.

In general, the identification of viruses as pathogens is based on Koch's postulates, which involve introducing the isolated viruses into healthy plants to reproduce the symptoms. However, viral diseases in passionfruit involve numerous factors, making it extremely complex to identify the pathogens. Specifically, disease development is influenced by the susceptibility of specific plant varieties to viruses, the virulence of the infecting virus/viruses, and environmental factors that promote symptom expression. For instance, PLV is known to show no or few signs of pathogenesis (Pares *et al*. 1997; Spiegel *et al*. 2007); conversely, symptoms have been reported to vary by origin of country. Although the cause is not clear, it is possible that different PLV strains exist. In addition, passionfruit has been reported to be infected by multiple viruses simultaneously. In Australia, passionfruit tip necrosis symptoms are thought to be caused by a mixed infection with passionfruit woody potyvirus (PWV) and CMV (Pares *et al*. 1985). Moreover, disease symptoms can differ from severe to minor, depending on the virus types. Among these, EAPV and EAPDV are known to negatively affect yields by causing fruit woodiness (Iwai *et al*. 2006; Riska *et al*. 2019). In particular, two EAPV types exist i.e., a fulminant form known as EAPV-AO which causes severe symptoms in passionfruit, and a mild form known as EAPV-IB. The abbreviations AO and IB stand for Amami Oshima and Ibusuki, respectively, indicating the regions where these strains were first identified.

There have been several cases of successful control of viral diseases in passionfruit. In Gifu Prefecture, Japan, a report indicated that the infection rate of CMV can be reduced by controlling aphids on passionfruit grown in the open air (Muramoto & Suzuki 2020). Another case involved the eradication of EAPV, where it was demonstrated that the virus could be controlled by removing old passionfruit seedlings and introducing healthy, virus-free seedlings. EAPV was first identified in 1986 on Amami Oshima Island, an isolated island in Kagoshima Prefecture. In 1992 and 1997, EAPV-AO was found to have spread across it as well (Iwai & Omatsu 2002). On Kikaijima, 30 km east of Amami Oshima Island, an isolated nursery of virus-free healthy seedlings was established, and cuttings propagated there were distributed to farmers to prevent the spread of the virus. As a result, a survey conducted from 2008 to 2010 revealed that EAPV-AO had significantly decreased to a few cases only in some areas of the island (Fukumoto *et al*. 2012; Iwai 2017).

In the present study, we investigated the cause of abnormal symptoms in passionfruit grown in Okinawa Prefecture from winter to spring by testing samples for a variety of previously reported viral diseases in Japan. Because the outbreak survey confirmed the high frequency of viral infections, we also examined the ef-

fect of suppressing the occurrence of abnormal symptoms by introducing virus-free seedlings.

MATERIALS AND METHODS

Sampling and observation of disease symptoms in the field

In the 2017 survey, leaf sampling was conducted from January to August in passionfruit fields on the two main islands of Okinawa, Miyako Island, and Ishigaki Island, mainly targeted plants with suspected viral symptoms. A total of 133 samples were collected from 84 fields.

Of these 133 samples, 104 samples were evaluated for field symptom observation and classification and the result was presented in Table 1 and Fig. 1. The remaining 29 samples- including 10 from Miyako Island, 16 from Ishigaki Island, and 3 from the southern region of Okinawa's main island- were excluded from symptom evaluation due to the absence of detailed field observation at those sites.

The plants sampled on the main island of Okinawa were classified according to the following symptoms (Fig. 1): A, leaf curling; B1, new shoot stunting; B2, leaf stunting; C, cracked leaf surface; D1, leaf yellowing; D2, yellowing between leaf veins; E, reduced vigor; and F, fruit malformation. The sampled leaves were placed in plastic bags and stored in the freezer at -80℃ until reverse transcription-polymerase chain reaction (RT-PCR) was performed.

In the 2022 survey, sampling was conducted in June in the southern part of the main island of Okinawa with a total of 58 samples collected from 21 fields.

In both surveys, virus detection was conducted solely by RT-PCR, and no virus isolation was performed from the field samples.

RNA extraction and RT-PCR analysis

The collected samples were subjected to RNA extraction for virus identification. Total RNA was isolated from both the young and old leaves of each plant using ISOGEN, reagent for RNA extraction (Nippon Gene Co., Ltd., Tokyo, Japan) according to the manufacturer's protocol. In brief, 1 mL of ISOGEN was added to 0.05 g of sample and grind. The solution was transferred into a 1.5-mL tube, added with 200 μL of chloroform, and shaken for 1 min. It was then centrifuged at 12,000 rpm (model 3780; Kubota, Tokyo, Japan) at 2℃ for 10 min,

Table 1. Identification of virus-like symptoms in passionfruit fields in Okinawa, Japan in 2017.[z]

	No. of virus-like symptoms[y]							
Survey location	A	B1	B2	C	D1	D2	E	F
The northern area of the main island	17	12	0	15	5	9	6	0
Motobu Town	2	1	0	1	0	0	0	0
Onna Village	15	11	0	14	5	9	6	0
The central area of the main island	1	1	3	0	0	0	0	3
Nishihara Town	1	0	0	0	0	0	0	0
Uruma City	0	1	3	0	0	0	0	3
The southern area of the main island	32	6	2	11	8	13	0	0
Itoman City	30	6	2	11	8	13	0	0
Nanjo City	1	0	0	0	0	0	0	0
Yaese Town	1	0	0	0	0	0	0	0
Total No.	50	19	5	26	13	22	6	3

[z] Symptom classification in Table 1 is based on visual observations of 104 passionfruit plants. Since multiple symptoms were often observed on a single plant, the total number of symptom entries amounts to 144.

[y] A: leaf curling; B1: new shoot stunting; B2: leaf stunting; C: leaf surface cracking; D1: yellowing of leaves; D2: yellowing between leaf veins; E: reduced vigor; and F: fruit malformation.

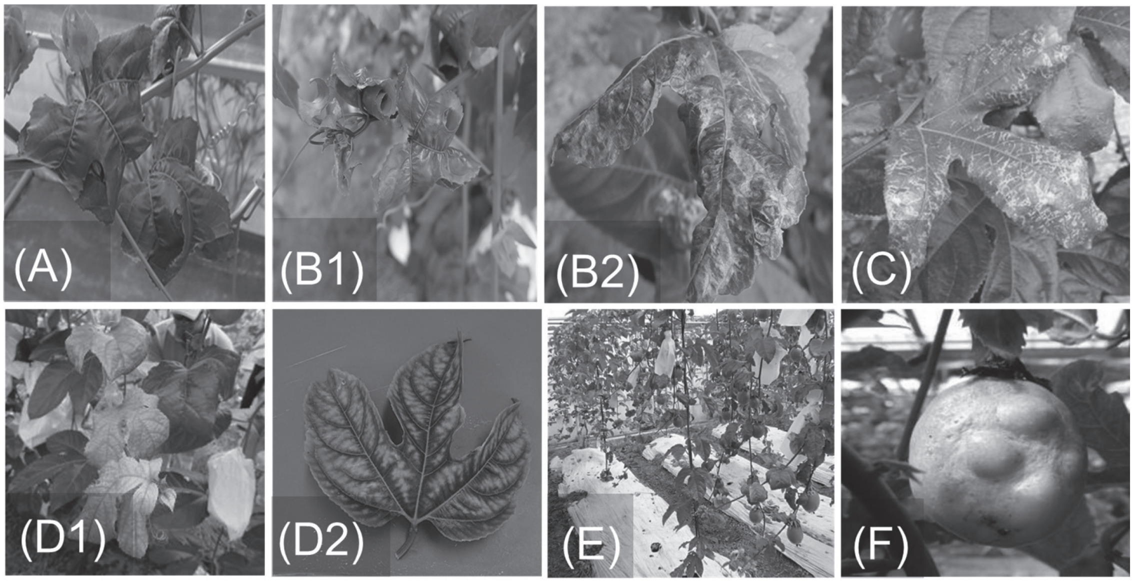

Fig. 1. Virus-like symptoms identified in passionfruit fields in Okinawa Prefecture, Japan: (A) leaf curling; (B1) new shoot stunting; (B2) leaf stunting; (C) leaf surface cracking; (D1) yellowing of leaves; (D2) yellowing between leaf veins; (E) reduced vigor; and (F) fruit malformation.

and 400 µL of the supernatant was transferred into a 1.5-mL tube, added with 400 µL of isopropanol, and shaken for 1 min. After centrifugation at 12,000 rpm at 2℃ for 10 min, the liquid in the 1.5-mL tube was discarded and 800 µL of 75% ethanol was added to dislodge the precipitate accumulated at the bottom by tapping the tube. After a second centrifugation at 12,000 rpm at 2℃ for 10 min, all the liquid in the 1.5-mL tube was carefully removed using a pipette, ensuring that the precipitate remained on the bottom. The tube was then inverted and left standing for 15 min to allow the evaporation of any remaining ethanol. Finally, 100 µL of RNase-free H_2O was added and the pellet was gently resuspended by tapping the tube to ensure complete dissolution. The obtained RNA was used as the template for reverse transcription reactions.

The presence of 6 viruses (BBWV-2, CMV, EAPDV, EAPV-AO, EAPV-IB, and PLV) was assessed via RT-PCR using previously published primers (Kondo *et al*. 2005; Tang *et al*. 2008; Fukumoto *et al*. 2012; Okada *et al*. 2017; Riska *et al*. 2019) which are listed in Table 2.

For the detection of EAPDV, cDNA was synthesized using the PrimeScript II 1st strand cDNA Synthesis kit (Takara Bio, Shiga, Japan) according to the manufacturer's instructions. The oligo(dT) primers listed in Table 2 were used for reverse transcription reactions. SapphireAmp Fast PCR Master Mix (Takara Bio, Shiga, Japan) was used for polymerase chain reaction (PCR), and reactions were carried out under the following conditions: one pre-denaturation cycle at 94℃ for 5 min; denaturation at 94℃ for 1 min; annealing at 55℃ for 1 min; extension at 72℃ for 2 min for 30 cycles in sets; and one extension cycle at 72℃ for 10 min.

For the other 5 viruses (BBWV-2, CMV, EAPV-AO, EAPV-IB, and PLV), RT-PCR amplifications were performed using the PrimeScript™ One Step RT-PCR Kit Ver. 2 (Dye Plus) (Takara Bio, Shiga, Japan). The thermal cycling conditions were as follows: pre-denaturation at 94℃ for 2 min, 25 cycles of denaturation for 30 s at 94℃, primer annealing for 30 s at 55℃, extension for 1 min at 72℃, and final extension at 72℃ for 10 min.

All PCR products were electrophoresed

Table 2. Primers used in this study for reverse transcription-polymerase chain reaction (RT-PCR) amplifications.

Target virus[z]	Name	Sequence 5′-3′	Size	Reference
BBWV-2	BBWVKMRM	TDGWDCCATCVAGICKCATTTT	320 bp	Kondo *et al*. (2005)
	BBWVVSSP	GTBTCDAGTGCTYTDGAAGG		
CMV	mul-CM-F2	GTTGCTTCCTGATTCAGTCAC	446 bp	Okada *et al*. (2017)
	mul-CM-R2	ACTACCAACTCAGCTCCCG		
EAPDV	oligo(dT)	CTAACTGTGGAAAGTCTTCC	800 bp	Riska *et al*. (2019)
	PV-F5	ACTCAATGCTGGGGACGCTC		
	PV-R7	CCCATACCAAGTAATGTGTG		
EAPV-AO	EAPVAO-F	TGCATGTCCTAGACCTC	1,201 bp	Fukumoto *et al*. (2012)
	AOIB200	T_{16}AGGACAAC		
EAPV-IB	EAPVIB-200	GACAAGAACGCCAGTTTG	1,245 bp	Fukumoto *et al*. (2012)
	AOIB200	T_{16}AGGACAAC		
PLV	PLV-F	CGAGACACACGCAAACGAA	523 bp	Tang *et al*. (2008)
	PLV-R	CAGCAAAGCAAAGACACGA		

[z] BBWV-2: Broad bean wilt virus-2; CMV: Cucumber mosaic virus; EAPDV: East Asian Passiflora distortion virus; EAPV-AO: East Asian Passiflora virus-AO; EAPV-IB: East Asian Passiflora virus-IB; PLV: Passiflora latent virus.

on a 1.5% agarose gel with GelRed (Biotium, Fremont, CA, USA) and irradiated with UV to confirm DNA amplification.

Identification of PLV in passionfruit using the DECS-C method

Total RNA was first extracted from 500 mg of leaves from a stunted passionfruit plant obtained from a seedling company (sampled in 2019). For comprehensive detection of both known and novel RNA viruses, the DECS-C method (dsRNA isolation, exhaustive cDNA amplification, and cloning-free next-generation sequencing) was employed as described by Yanagisawa *et al*. (2016). Briefly, dsRNA was extracted using a plant viral dsRNA enrichment kit (MBL, Aichi, Japan) according to the manufacturer's protocol. The TransPlex Whole Transcriptome Amplification (WTA) Kit (Sigma, St. Louis, MO, USA) was used to reverse-transcribe the extracted dsRNA into cDNA employing the supplied random primer and then to amplify the obtained cDNA following the manufacturer's protocol. PCR amplification was performed using ExTaq DNA polymerase (Takara Bio, Shiga, Japan) for a total of 20 cycles. The WTA products were purified using AMPure XP (Beckman Coulter, Brea, CA, USA). In total, 100 ng of WTA products was used to construct the sequence library via the TruSeq RNA Library Prep Kit (Illumina Inc., San Diego, CA, USA), and sequencing was performed on a MiSeq system (Illumina Inc., San Diego, CA, USA). The CLC Genomics Workbench ver. 11.0 (CLC Bio, Tokyo, Japan) was used for the analysis of sequence data. The mapping analysis was performed using the complete genomes of all viruses and viroids registered in the NCBI. The isolate obtained from a stunted seedling by the DECS-C method is hereafter referred to as the "PLV-OP isolate."

Plant growth conditions for virus inoculation

Plants of *Chenopodium quinoa* Willd. were grown in pots in a growth room at 25℃ under a 16-h photoperiod, while plants of *P. edulis* (passionfruit) were grown in a greenhouse. Three-week-old quinoa plants were used for inoculation with PLV-OP isolate and subsequent isolation. After inoculation, the plants were placed under fluorescent lights in a cool, air-conditioned laboratory for the observation of symptom development.

PLV inoculation and isolation

For the isolation of PLV, the PLV-OP iso-

late- originally obtained from a stunted passionfruit seedling and identified through DECS-C analysis- was used throughout. Quinoa plants were first inoculated with the sap of PLV-OP-infected passionfruit leaves using carborundum following the standard protocol. Then, single lesion isolation was performed through six repeated inoculations on quinoa plants. Finally, 10 g of leaves were collected for viral particle purification. The viral particles were partially purified as previously described in Atsumi *et al.* (2015). PLV-specific proteins were confirmed by SDS-PAGE using 5–20% e-PAGEL (ATTO Corp., Tokyo, Japan) and TaKaRa CBB Protein Safe Stain (Takara Bio, Shiga, Japan).

Testing PLV transmission by aphids

Cotton aphids (*Aphis gossypii* Glover) and green peach aphids (*Myzus persicae* Sulzer) raised cumulatively on healthy sweet pepper (*Capsicum annuum* L.) and eggplant (*Solanum melongena* L.) plants were fasted in a beaker for 2 h prior to allowing PLV acquisition via sucking. Specifically, using a brush, the fasted aphids were transferred to fresh upper leaves of passionfruit collected from plants infected with the PLV-OP isolate and were allowed to suck the sap for approximately 10 min. Then, a total of 10 or 20 aphids per plant were used for the inoculation of healthy passionfruit seedlings. The day after inoculation, Starkle® granules (1% granular dinotefuran, Hokko Chemical Co., Ltd., Tokyo, Japan) were applied at the base of each plant and, once the aphids were confirmed dead, the seedlings were moved into a glass room for the observation of the development of disease symptoms.

Testing PLV transmission via pruning shears

A passionfruit vine infected with the PLV-OP isolate was cut using pruning shears; then, the same shears were used to prune a healthy passionfruit seedling vine with approximately two to three true leaves. The pruned sapling was placed in a glasshouse and, after 1 mo, was subjected to both the examination of disease symptoms and testing for PLV-OP infection by RT-PCR. The experiment was conducted twice, in 2019 and 2020. In the 2019 trial, 18 replicates were performed, while 35 replicates were conducted in 2020, resulting in a total of 53 replicates over the two years.

RESULTS

Virus-like symptoms observed in passionfruit fields during the 2017 survey

In Okinawa Prefecture, abnormal virus-like symptoms had been observed in passionfruit during the winter/spring season prior to 2017 and had become a problem. Thus, this prompted a survey of virus diseases in passionfruit fields in this region in 2017. The four most common symptoms identified across Okinawa Island were (A) leaf curling (50 samples), (C) leaf surface cracking (26 samples), (D2) yellowing between leaf veins (22 samples) , and (B1) new shoot stunting (19 samples) (Table 1, Fig. 1). In contrast, the lowest incidence was observed for (F) fruit malformation (3 samples), followed by (B2) leaf stunting (5 samples) and (E) reduced vigor (6 samples). In total, 144 symptom observations were recorded from 104 passionfruit plants evaluated for visible abnormalities during this survey. This discrepancy reflects the fact that multiple symptoms were often observed on a single plant.

Distribution of passionfruit viral outbreaks in Okinawa Prefecture

In the 2017 survey, a total of 84 passionfruit fields (133 samples) in Okinawa Prefecture were investigated for the presence of virus-like infection in the leaves via RT-PCR tests. These fields were distributed as follows: 28 fields (39 samples) in the northern area of the main island, 3 fields (8 samples) in the central area, 36 fields (60 samples) in the southern area, 5 fields (10 samples) in Miyakojima Island, and 12 fields (16 samples) in the Yaeyama Islands. EAPDV was detected in 7 samples (2.4%, 5.3%) from 2 fields, EAPV-AO in 2 samples (1.2%, 1.5%)

from 1 field, and PLV in 107 samples (85.7%, 80.5%) from 72 fields (Table 3). Cases of infection with BBWV-2, CMV, and EAPV-IB were not confirmed. While symptoms in many infected plants were minor and had negligible impacts on cultivation, yet severe virus-like symptoms (e.g., fruit deformities) were observed in some plants in Uruma City and Ishigaki City. These cases involved overlapping infections: EAPDV and PLV in Uruma City whereas EAPDV, EAPV-AO and PLV in Ishigaki City. These observations demonstrated that passionfruit can be co-infected with three or more viruses. The survey also showed the frequent infection of passionfruit with PLV in Okinawa Prefecture and the presence, for the first time, of EAPV-AO in the area, highlighting the need to conduct periodic surveys of passionfruit viral outbreaks and to be vigilant for the emergence of new viral infections.

Distribution of PLV-infected passionfruit in Okinawa Prefecture in 2022

In 2022 survey, a total of 21 passionfruit fields (58 samples) in the southern part of the main island of Okinawa were investigated for PLV infection in the leaves via RT-PCR. The results showed that, since 2017, the incidence of PLV infections in Okinawa Prefecture markedly decreased from the previously high field rate of 85.7%. This was due to efforts to inform passionfruit farmers about the disease and to the introduction of virus-free seedlings obtained from Kagoshima Prefecture. Of the 15 fields planted with virus-free seedlings, three became infected with PLV which corresponded to an incidence of 20%. The survey of these 15 fields showed that 6 out of 39 (15.4%) samples were PLV-positive. Alternatively, of the six fields where virus-free seedlings were not used, five became infected with PLV, which corresponded to an incidence of 83.3%. The survey of these six fields showed that 15 out of 19 (78.9%) samples were PLV-positive (Table 4). For viruses other than PLV (i.e., BBWV-2, CMV, EAPV-AO, EAPV-IB, and EAPDV), no infections were identified in 21 fields (58 samples). This suggested that the spread of PLV in Okinawa Prefecture and the distribution of diseased seedlings were the main causes of the widespread occurrence of passionfruit virus outbreaks in the region. However, three of the 15 fields planted with virus-free seedlings were found to be re-infected with PLV despite the introduction of such seedlings. Based on interviews with growers after this survey, they confirmed that they had planted a mixture of self-propagated and virus-free seedlings. This suggested that either transmission by sap-sucking aphids or by juice remained on pruning shears may have occurred in the field.

Identification of PLV from a passionfruit nursery using the DECS-C method

Based on the 2017 survey result, PLV was considered as the primary pathogen, thus attempts were made to characterize and isolate it from diseased passionfruit. Comprehensive RNA virus detection techniques (i.e., DECS-C) were applied to passionfruit seedlings obtained from a seedling company that exhibited leaf morphological abnormalities (Fig. 2A). Deep sequencing yielded a total of 508,110 sequence reads. Mapping analysis was performed using reference sequences from approximately 7,000 viral genomes which resulted in a total of 35,369 reads being mapped to the reference genome of PLV (Fig. 3). For all the other viruses examined, the number of sequence reads mapped to their genomes was not sufficient to ensure reliable results. The consensus genomic sequence obtained from deep sequencing and mapping analysis showed 91.93% identity with the PLV isolate DSMZ PV-0222 (MT723990). Based on the result of sap inoculation on quinoa plants, the PLV isolate identified in this study caused necrotic spots on the inoculated leaves (Fig. 2B).

PLV inoculation and isolation

Single-lesion isolation was repeated 6 times before the systemically infected crude viral particles were subjected to purification. The crude viral particle fraction was separated by SDS-PAGE and stained with Coomassie

Table 3. Infected fields and rates of six viruses (in samples) already occurring in Japan in passionfruit fields in Okinawa, Japan, in 2017.

	BBWV-2[z]		CMV		EAPDV		EAPV-AO		EAPV-IB		PLV	
Survey location	Fields	Samples	Fields	Samples	Fields	Samples	Fields	Samples	Fields	Samples	Fields	Samples
The northern area of the main island	0/28[y]	0/39	0/28	0/39	0/28	0/39	0/28	0/39	0/28	0/39	26/28	33/39
Motobu Town	0/1	0/4	0/1	0/4	0/1	0/4	0/1	0/4	0/1	0/4	1/1	3/4
Onna Village	0/27	0/35	0/27	0/35	0/27	0/35	0/27	0/35	0/27	0/35	25/27	30/35
The central area of the main island	0/3	0/8	0/3	0/8	1/3	4/8	0/3	0/8	0/3	0/8	3/3	7/8
Nishihara Town	0/1	0/2	0/1	0/2	0/1	0/2	0/1	0/2	0/1	0/2	1/1	1/2
Uruma City	0/2	0/6	0/2	0/6	1/2	4/6	0/2	0/6	0/2	0/6	2/2	6/6
The southern area of the main island	0/36	0/60	0/36	0/60	0/36	0/60	0/36	0/60	0/36	0/60	35/36	56/60
Itoman City	0/33	0/54	0/33	0/54	0/33	0/54	0/33	0/54	0/33	0/54	32/33	50/54
Nanjo City	0/1	0/3	0/1	0/3	0/1	0/3	0/1	0/3	0/1	0/3	1/1	3/3
Yaese Town	0/2	0/3	0/2	0/3	0/2	0/3	0/2	0/3	0/2	0/3	2/2	3/3
Miyakojima Island	0/5	0/10	0/5	0/10	0/5	0/10	0/5	0/10	0/5	0/10	3/5	5/10
Miyakojima City	0/5	0/10	0/5	0/10	0/5	0/10	0/5	0/10	0/5	0/10	3/5	5/10
Yaeyama Islands	0/12	0/16	0/12	0/16	1/12	3/16	1/12	2/16	0/12	0/16	5/12	6/16
Ishigaki City	0/12	0/16	0/12	0/16	1/12	3/16	1/12	2/16	0/12	0/16	5/12	6/16
Sum	0/84	0/133	0/84	0/133	2/84	7/133	1/84	2/133	0/84	0/133	72/84	107/133
Incidence rate	0.0	0.0	0.0	0.0	2.4	5.3	1.2	1.5	0.0	0.0	85.7	80.5

[z] BBWV-2: Broad bean wilt virus 2; CMV: Cucumber mosaic virus; EAPDV: East Asian Passiflora distortion virus; EAPV-AO: East Asian Passiflora virus-AO; EAPV-IB: East Asian Passiflora virus-IB; and PLV: Passiflora latent virus.

[y] No. of virus infections/No. of reverse transcription-polymerase chain reaction (RT-PCR) tests.

Table 4. Number of infected passionfruit fields and rates of PLV infection in samples in Okinawa Prefecture, Japan, in 2022.

	No. of fields		No. of samples	
Seedling	Positive	Negative	Positive	Negative
Virus-free	3/15[z] (20.0)[y]	12/15 (80.0)	6/39 (15.4)	33/39 (84.6)
Not virus-free	5/6 (83.3)	1/6 (16.7)	15/19 (78.9)	4/19 (21.1)

[z] No. of PLV-infected fields (samples) by reverse transcription-polymerase chain reaction (RT-PCR)/No. of surveyed fields (samples).
[y] Parentheses indicate percentages.

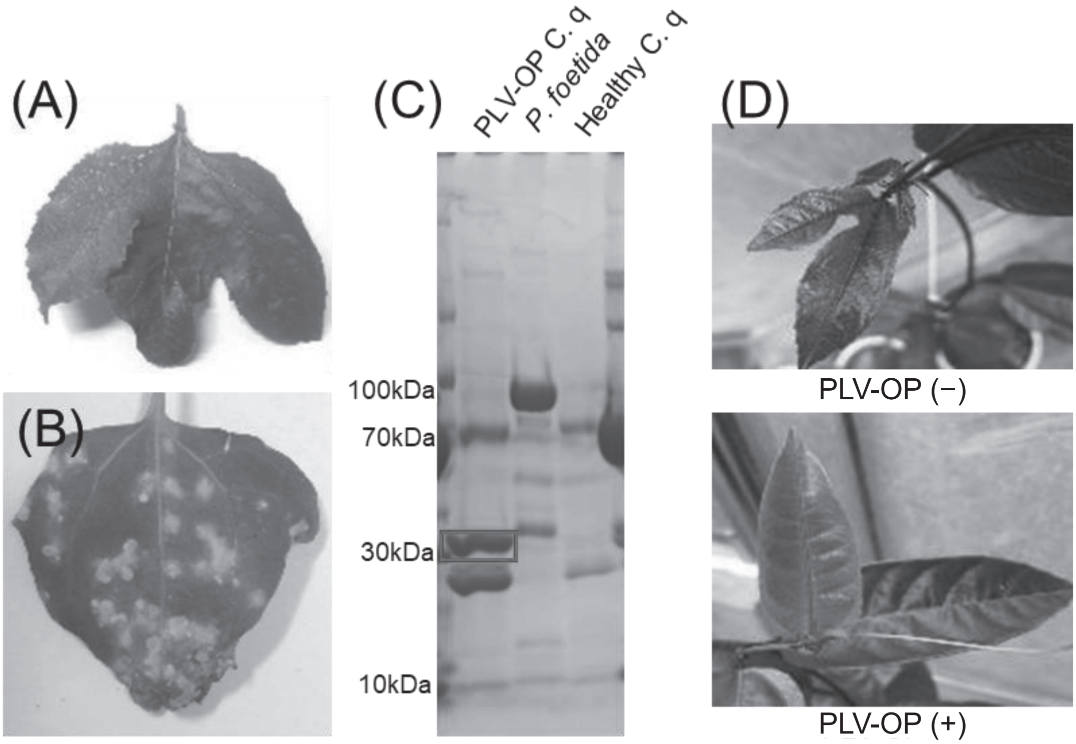

Fig. 2. PLV-OP isolated from a diseased passionfruit seedling. (A) Virus-like symptoms observed on the leaf of a diseased passionfruit seedling obtained from a commercial seedling supplier in Okinawa. (B) Necrotic spots on a leaf inoculated with PLV-OP. (C) Protein bands obtained from specific fractions during the purification of viral particles extracted from infected passionfruit plants. (D) Healthy appearance of passionfruit plants inoculated with PLV-OP (+) compared to non-inoculated plants PLV-OP (−).

Brilliant Blue (CBB) that revealed a band of approximately 30 kDa. This band was estimated to represent the coat protein of PLV (Fig. 2C). The isolated virus was subsequently confirmed by RT-PCR and designated as the Okinawa passionfruit isolate of PLV (PLV-OP). Next, mechanical inoculation tests were conducted to evaluate its pathogenicity. The virus was inoculated onto the mature leaves of passionfruit plants using a crude extract containing PLV. One week after inoculation, PLV was detected in the newly emerged, non-inoculated leaves by RT-PCR. However, no visible symptoms developed and the infected plants grew similarly to healthy controls (Fig. 2D). For grafting experiments, the virus-free seedlings were grafted with scions obtained from PLV-infected passionfruit plants with abnormal symptoms,

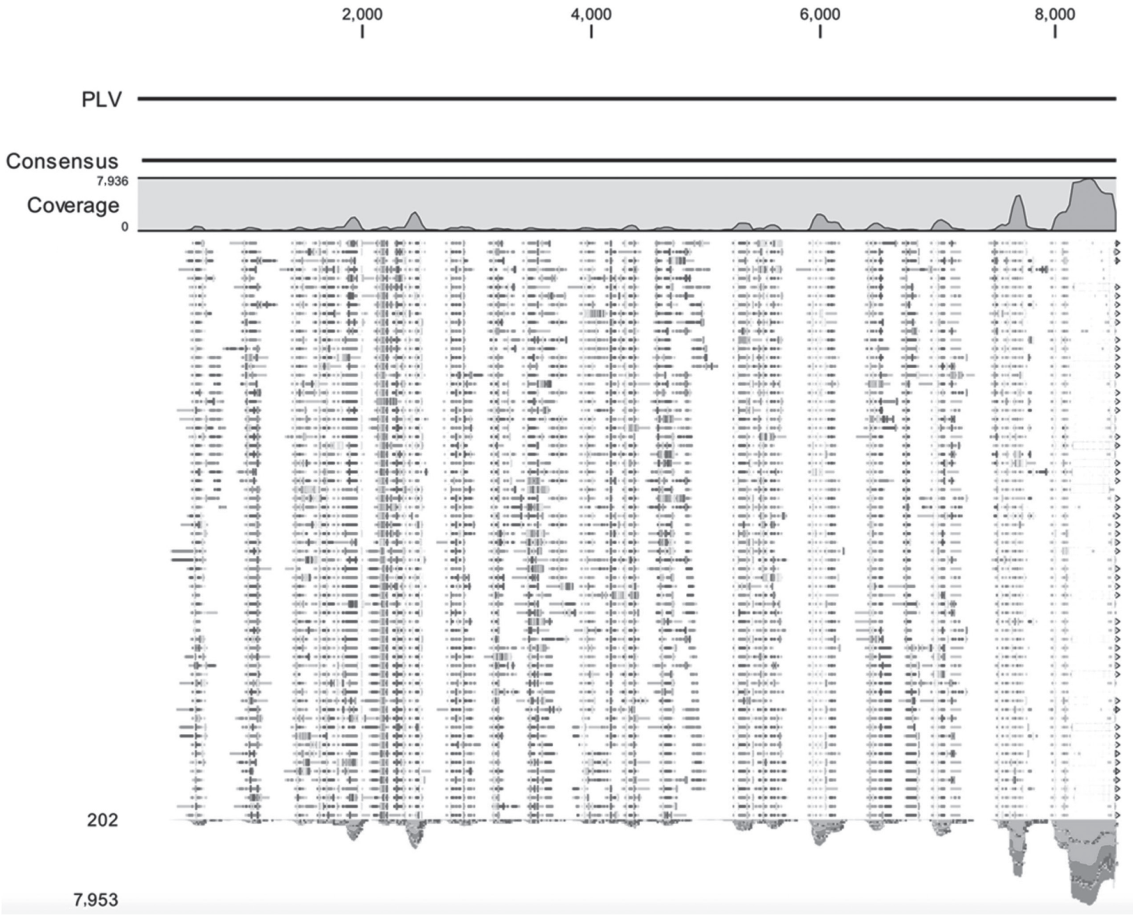

Fig. 3. Whole Passiflora latent virus (PLV) genome covered by sequence reads derived from dsRNA extracted from diseased passionfruit. The numbers indicate the depth of 35,369 mapped-reads analyzed using the CLC Genomics Workbench.

results showed that no reproduction of the specific symptoms previously observed in passionfruit during the winter/spring season in Okinawa Prefecture. Sap extracted from symptomatic tissues was used to inoculate healthy passionfruit seedlings yielded no similar symptoms as that observed in fields.

Testing PLV transmission by aphids

Propagation tests were conducted to investigate how PLV spreads in passionfruit fields. The ability of *A. gossypii* and *M. persicae* to transmit PLV to passionfruit plants was assessed. For *A. gossypii*, 10 or 20 aphids were allowed to feed on passionfruit plants infected with the PLV-OP isolate and subsequently transferred to virus-free passionfruit plants, no symptoms were observed in the recipient plants. However, RT-PCR detected the presence of PLV in 22.2% of the plants inoculated with 10 aphids and 16.7% of those inoculated with 20 aphids. In contrast, when *M. persicae* were used in the same manner, neither symptoms nor PLV infection were detected in the recipient plants (Table 5).

Testing PLV transmission via pruning shears

Tests were conducted to assess PLV-OP transmission in passionfruit plants via pruning

Table 5. Transmission of Passiflora latent virus (PLV) by aphids.

Aphid	Feeding period[z]	No. of insects[y]	Symptomatic plant[x]	Positive reaction[w]
Aphis gossypii	10 min	10	0/9 (0.0)[v]	2/9 (22.2)
A. gossypii	10 min	20	0/6 (0.0)	1/6 (16.7)
Myzus persicae	10 min	15	0/6 (0.0)	0/6 (0.0)

[z] After being fasted for 2 h, the aphids were allowed to feed on infected leaves for different periods and then transferred to the test plants, on which they continued to feed overnight.
[y] No. of insects used for inoculation per plant.
[x] No. of symptomatic plants/No. of inoculated plants.
[w] No. of PLV-positive plants by reverse transcription-polymerase chain reaction (RT-PCR)/No. of inoculated plants.
[v] Parentheses indicate percentages.

shears. In the 2019 and 2020 surveys, no symptoms were observed, although RT-PCR results showed infection rates of 94.4% and 8.6%, respectively. Throughout the 2-year survey, the average rate of PLV infection by pruning shears was confirmed at 37.7%, although no viral symptoms were identified in any of the samples (Table 6).

Table 6. Transmission of Passiflora latent virus (PLV) by contaminated pruning shears.

Experiment year	Symptomatic plant[z]	Positive reaction[y]
2019	0/18 (0.0)[x]	17/18 (94.4)
2020	0/35 (0.0)	3/35 (8.6)
Average	0/53 (0.0)	20/53 (37.7)

[z] No. of symptomatic plants/No. of inoculated plants.
[y] No. of PLV-positive plants by reverse transcription-polymerase chain reaction (RT-PCR)/No. of inoculated plants.
[x] Parentheses indicate percentages.

DISCUSSION

Based on the 2017 field survey, PLV was detected in 107 out of 133 samples (80.5%), making it the most prevalent virus in Okinawa Prefecture. However, symptom observation did not show a consistent pattern associated with PLV infection alone. As indicated in Fig. 1 and Table 1, PLV-positive plants exhibited various symptoms including leaf curling, interveinal chlorosis, new shoot stunting, and fruit malformation. These symptoms were generally diverse, mild to severe and non-specific, suggesting that PLV infection alone may not fully account for the abnormalities observed in the field.

Furthermore, mechanical inoculation using purified PLV-OP isolate (Fig. 2D) did not reproduce visible symptoms in passionfruit plants, even though systemic infection was confirmed by RT-PCR. This reinforces the hypothesis that PLV may behave as a latent or weakly pathogenic virus, with visible symptom expression dependent on environmental conditions, host factors, or viral co-infection.

Supporting this interpretation, Table 3 shows that severe symptoms such as fruit deformation and shoot distortion were observed in Uruma City and Ishigaki City, where PLV co-occurred with EAPDV and/or EAPV-AO. These cases suggest that co-infection may intensify symptom severity, a phenomenon commonly reported among Carlaviruses. Therefore, effective disease diagnosis and management should consider both viral prevalence and the possibility of co-infection in affected fields.

The survey results indicated a possible correlation between the large-scale growth abnormalities in passionfruit cultivated in Okinawa Prefecture and PLV infection. However, in this study, the back-inoculation test using the PLV-OP isolate did not reproduce the pathological symptoms. As a result, Koch's postulates for confirming the pathogen's role were not fulfilled. Viruses belonging to the genus *Carlavirus*, such as PLV, often require co-infection with other viruses to manifest symptoms. For example, Carnation latent virus (CLV) in Japanese carnations typically remains asymptomatic but causes symptoms when co-infected with Carnation vein mottle virus (Morita &

Horie 1998). Similarly, Lily symptomless virus in lilies remains latent but leads to guttate lesions when co-infected with CMV (Nishi *et al*. 2008). Therefore, it is possible that PLV alone does not cause severe damage in passionfruit but may do so when combined with other viruses. In this study, no other viruses were detected in the PLV-infected passionfruit plants using the DECS-C method. Previous studies on PLV's virulence in passionfruit (Bao *et al*. 2023; Choi & Ju 2023) have shown varying results some with strong virulence while others with minimal or no impact. These discrepancies suggest that different PLV strains might contribute to the varying symptoms observed in passionfruit. Moreover, the pronounced symptoms during winter and spring hint at the significant influence of environmental factors on disease development.

In another region of Japan, Gifu Prefecture, a survey on viral diseases on passionfruit was conducted similar to the study in Okinawa Prefecture. The survey in Gifu Prefecture revealed that 72.1% of passionfruit plants were infected with PLV (Muramoto & Suzuki 2018). Additionally, the survey reported a high incidence of CMV outbreaks which were not detected in Okinawa Prefecture. This difference in CMV incidence between the two prefectures may be attributed to differences in cultivation methods. In Okinawa Prefecture, passionfruit is cultivated indoors due to high rainfall which decreases susceptibility to pests and diseases. In contrast, in Gifu Prefecture, passionfruit is grown in open fields where aphid infestations are more likely to occur (Kumamoto *et al*. 2017). In Gifu Prefecture, CMV infection rates are reduced when aphids are controlled. Furthermore, because CMV causes clear leaf atrophy symptoms, it is unlikely that infected seedlings (and thus the virus) are introduced into fields (Muramoto & Suzuki 2020). Therefore, it is inferred that CMV contamination occurs after seedlings are planted, primarily through aphid transmission. High CMV infection rates have been observed in Gifu Prefecture, where aphids easily infest passionfruit in open fields. Whereas, no cases of CMV were detected in Okinawa Prefecture where passionfruit is cultivated indoors. Based on these findings, the present study also examined the possibility of PLV transmission via two aphid species as well as sap-contaminated pruning shears. The results indicated that *A. gossypii* transmitted PLV in a non-persistent manner. Non-persistent transmission means that the virus is spread by aphids during short feeding sessions, without the virus being retained for long periods. Viruses belonging to the genus *Carlavirus*, including PLV, have been reported to be transmitted in this manner. Specifically, Omatsu *et al*. (2004) confirmed non-persistent transmission of EAPV by the sowthistle aphid (*Nogeshi-fukure-aburamushi*; *Hyperomyzus carduellinus*), cotton aphid, and peach aphid.

Among the studies conducted on the transmission of passion fruit viruses by aphids, Parry *et al*. (2004) and Yonaha *et al*. (1979) reported infections with Passiflora virus Y (PaVY) via cotton aphids and the non-persistent transmission of CMV by peach aphids, respectively. These reports indicate that aphid control is important for mitigating the spread of passionfruit virus diseases including PLV. However, in recent years, PLV infections have been confirmed in many passion fruit farms in Okinawa Prefecture, although these were all within greenhouse environments where aphid infestations have been rarely reported. Therefore, it is unlikely that aphids are an important route of PLV transmission in this prefecture. However, this study showed that sap-contaminated pruning shears represented an effective means of PLV transmission, especially in passionfruit which requires frequent pruning. This suggested that once the virus enters a field, it can spread across it through cultivation management practices.

In addition, measures against passionfruit virus disease have been thoroughly promoted by conducting RT-PCR tests on samples obtained from seedling suppliers in Okinawa Prefecture. These efforts, which have been ongoing since 2019, aim to identify and prevent

the spread of infected seedlings. This approach complements the findings of the present study, as it highlights the importance of ensuring the distribution of virus-free seedlings to mitigate disease outbreaks.

In summary, the present study revealed widespread viral infections in passionfruit fields in Okinawa Prefecture, with PLV identified as the major virus transmitted via aphids and contaminated pruning shears. The introduction of virus-free seedlings significantly reduced the incidence of PLV during the growing season, indicating that the distribution of infected seedlings was the primary source of infections. However, when virus-free seedling fields are adjacent to fields with non-virus-free seedlings, or in fields where virus-free and non-virus-free seedlings are grown together, controlling aphids and disinfecting pruning shears become essential. Comprehensive and integrated control strategies will be crucial for managing passionfruit virus diseases effectively in the future.

ACKNOWLEDGMENTS

We sincerely thank the many Okinawa Prefectural Agricultural Extension Officers who supported the outbreak surveys conducted in 2017. Our gratitude also extends to Dr. H. Iwai of Kagoshima University for providing valuable information on EAPDV-positive controls and RT-PCR cycle parameters.

REFERENCES

Atsumi, G., R. Tomita, T. Yamashita, and K. T. Sekine. 2015. A novel virus transmitted through pollination causes ring-spot disease on gentian (*Gentiana triflora*) ovaries. J. Gen. Virol. 96:431–439. doi:10.1099/vir.0.071498-0

Bao, S., F. Y. Ge, X. Y. Li, and B. Bo. 2023. First report of Passiflora latent virus in passion fruit (*Passiflora edulis*) in China. Plant Dis. 107:3326. doi:10.1094/PDIS-05-23-0896-PDN

Choi, M. K. and H. J. Ju. 2023. First report of Passiflora latent virus infecting passion fruit (*Passiflora edulis*) in South Korea. Plant Dis. 107:2893. doi:10.1094/PDIS-01-23-0051-PDN

Fukumoto, T., M. Nakamura, M. Rikitake, and H. Iwai. 2012. Molecular characterization and specific detection of two genetically distinguishable strains of East Asian Passiflora virus (EAPV) and their distribution in southern Japan. Virus Genes 44:141–148. doi:10.1007/s11262-011-0676-7

Iwai, H. 2017. Studies on the diagnosis and genomic analysis of the plant viral diseases which occur in southern Japan. Jpn. J. Phytopathol. 83:124–128. (in Japanese) doi:10.3186/jjphytopath.83.124

Iwai, H. and N. Omatsu. 2002. Passionfruit woodiness virus in Japan. Shokubutsu Boueki. 56:110–113. (in Japanese)

Iwai, H., Y. Yamashita, N. Nishi, and M. Nakamura. 2006. The potyvirus associated with the dappled fruit of *Passiflora edulis* in Kagoshima Prefecture, Japan is the third strain of the proposed new species East Asian Passiflora virus (EAPV) phylogenetically distinguished from strains of *Passion fruit woodiness virus*. Arch. Virol. 151:811–818. doi:10.1007/s00705-005-0659-x

Kobayashi, Y. O., Y. Mikoshiba, Y. Honda, and T. Omura. 2004. Detection of *Broad bean wilt virus 1* and *Broad bean wilt virus 2* in Japan. Annu. Rep. Kanto Tosan. Plant Prot. Soc. 51:43–48. (in Japanese with English abstract) doi:10.11337/ktpps1999.2004.43

Kondo, T., S. I. Fuji, K. Yamashita, D. K. Kang, and M. U. Chang. 2005. *Broad bean wilt virus 2* in yams. J. Gen. Plant Pathol. 71:441–443. doi:10.1007/s10327-005-0236-x

Kumamoto, O., A. Higashi, T. Suzuki, N. Suzuki, M. Hashimoto, T. Kondo, and H. Higuchi. 2017. Current situation of passion fruit production and the latest techniques. Res. Trop. Agric. 10:7–17. (in Japanese) doi:10.11248/nettai.10.7

Morita, H. and H. Horie. 1998. Carnation virus disease. p. 651. *in*: Plant Diseases in Japan. (Kishi, K. ed.) Zenkoku Noson Kyoiku Kyokai. Tokyo, Japan. 1276 pp. (in Japanese)

Muramoto, Y. and T. Suzuki. 2018. Situation of virus infection in passion fruit in Gifu Prefecture and RT-PCR method for detection of two viruses. Hortic. Res. (Japan) 17(Suppl. 1):87. (in Japanese)

Muramoto, Y. and T. Suzuki. 2020. Occurrence and control of virus diseases of passion fruit in Gifu Prefecture. Jpn. J. Phytopathol. 86:60. (in Japanese) doi:10.3186/jjphytopath.86.44

Nakasato, K., S. Fujioka, Y. Sugawara, T. Ono, T. Nishio, and S. Tsuda. 2020. First detection of two potyviruses, uraria mosaic virus and passiflora mosaic virus Y, from passionfruit in Japan. J. Gen. Plant Pathol. 86:401–404. doi:10.1007/s10327-020-00932-4

Nishi, N., T. Muta, K. Miyaji, N. Omatsu, R. Hakuzan, I. Shinyashiki, Y. Tasaki, … H. Iwai. 2008. An etiological survey and serological diagnosis of reddish-brown streaks on the stem of Japanese Easter Lily “Hinomoto” in Okinoerabu Island. Kyushu Pl. Prot. Res. 54:39–43. (in Japanese with English abstract). doi:10.4241/kyubyochu.54.39

Okada, R., S. Numata, and S. Nishimiya. 2017. Development of a batch diagnostic technique by multiplex RT-PCR of eight viruses infecting *Capsicum annuum*. Ann. Rept Ibaraki Plant Prot. Soc. 56:15–20. (in Japanese)

Omatsu, N., H. Iwai, O. Setokuchi, and K. Arai. 2004. Immigrating aphid species and their importance as vectors of passionfruit woodiness virus in the fields of Amami Oshima Island, Japan. Mem. Fac. Agric. Kagoshima Univ. 39:1–5.

Pares, R. D., L. V. Gunn, E. N. Keskula, A. B. Martin, and D. S. Teakle. 1997. Occurrence of Passiflora latent carlavirus in cultivated and wild Passiflora species in Australia. Plant Dis. 81:348–350. doi:10.1094/PDIS.1997.81.4.348

Pares, R. D., A. B. Martin, and R. D. Fitzell. 1985. Virus-induced tip necrosis of passionfruit (*Passiflora edulis* Sims). Australas. Plant Pathol. 14:76–78. doi:10.1071/APP9850076

Parry, J. N., R. I. Davis, and J. E. Thomas. 2004. Passiflora virus Y, a novel virus infecting *Passiflora* spp. in Australia and the Indonesian Province of Papua. Australas. Plant Pathol. 33:423–427. doi:10.1071/AP04042

Riska, Y. Sato, K. Inudo, M. Nakamura, T. Fukumoto, T. Takushi, ... H. Iwai. 2019. East Asian *Passiflora* distortion virus: A novel potyvirus species causing deformation of passionfruits in Japan. J. Gen. Plant Pathol. 85:221–231. doi:10.1007/s10327-019-00842-0

Spiegel, S., M. Zeidan, I. Sobolev, Y. Beckelman, V. Holdengreber, Y. Tam, … A. Gera. 2007. The complete nucleotide sequence of Passiflora latent virus and its phylogenetic relationship to other carlaviruses. Arch. Virol. 152:181–189. doi:10.1007/s00705-006-0833-9

Tang, J., G. R. G. Clover, B. J. R. Alexander, and B. D. Quinn. 2008. First report of *Passiflora latent virus* in banana passionfruit (*Passiflora tarminiana*) in New Zealand. Plant Dis. 92:486. doi:10.1094/PDIS-92-3-0486C

Watanabe, M., C. Noda, T. Maoka, T. Usugi, and K. T. Natsuaki. 1997. Occurrence of *Passiflora latent virus* in Japan. Jpn. J. Phytopathol. 63:194. (in Japanese) doi:10.3186/jjphytopath.63.193

Yanagisawa, H., R. Tomita, K. Katsu, T. Uehara, G. Atsumi, C. Tateda, ... K. T. Sekine. 2016. Combined DECS analysis and next-generation sequencing enable efficient detection of novel plant RNA viruses. Viruses 8:70. doi:10.3390/v8030070

Yonaha, T., T. Hiraiwa, and T. Ooisi. 1993. Broad bean wilt virus isolated from passionfruit. Jpn. J. Phytopathol. 59:335. (in Japanese) doi:10.3186/jjphytopath.59.335

Yonaha, T., M. Tamori, S. Yamanoha, and T. Nakasone. 1979. Studies on passionfruit virus diseases in Okinawa: 1. Cucumber mosaic virus isolated from diseased *Passiflora edulis* and *Passiflora foetida* plants. Sci. Bull. Coll. Agric. Univ. Ryukyus. 26:29–38. (in Japanese with English abstract)

Yonemoto, Y. and T. Kondo. 2020. “What is passionfruit?” p.23–42. *in*: New Specialty Crop Series: Passionfruit—From Professionals to Home Growers (Yonemoto, Y. and T. Kondo, eds.) Nosan Gyoson Bunka Kyokai. Tokyo, Japan. 136 pp. (in Japanese)

日本沖繩百香果病毒調查：百香果潛隱病毒的流行、傳播及利用去病毒苗達有效防治

Atsushi Ajitomi[1,*] Akari Saito[2] Shinji Kawano[3] Ken-taro Sekine[4,*]

摘要

Ajitomi, A., A. Saito, S. Kawano, and K. T. Sekine. 2025。日本沖繩百香果病毒調查：百香果潛隱病毒的流行、傳播及利用去病毒苗達有效防治。台灣農業研究 74(3):275–289。

在日本沖繩縣，冬春季節種植的百香果出現新葉枯萎、植株活力下降等徵狀的栽培問題。2017 年，調查 84 個田區而確認了疑似病毒病的徵狀，並以 reverse transcription-polymerase chain reaction (RT-PCR) 進一步調查日本已報告的 6 種特定病毒的疫情。結果顯示，最常見的 3 種病徵是成年葉片捲曲、葉面開裂及葉脈間黃化。在檢測的 6 種病毒中，東亞百香果皺縮病毒 (East Asian Passiflora distortion virus; EAPDV)、東亞百香果病毒 -AO (East Asian Passiflora virus-AO; EAPV-AO) 與百香果潛隱病毒 (Passiflora latent virus; PLV) 的發生率分別為 2.4%、1.2% 及 85.7%，而蠶豆萎凋病毒 -2 (Broad bean wilt virus-2; BBWV-2)、胡瓜嵌紋病毒 (Cucumber mosaic virus; CMV) 均未檢測到。感染 EAPDV 與 EAPV-AO 的植物表現出葉片皺縮與果實畸形的典型徵狀，而感染 PLV 的植物則表現出其他非特異性徵狀。根據調查結果，PLV 被確定為主要病原。此病毒是從受感染的百香果中所分離而得，可導致奎藜出現病徵，但不會在百香果引起病徵。PLV 可透過昆蟲媒介 (例如棉蚜) 以及修枝剪所殘留的汁液傳播。此外，由於在從種苗供應商的百香果幼苗中檢測到了 PLV，因此從 2019 年開始，沖繩縣採取了供應已確認不含 PLV 的幼苗措施。此後，PLV 的發生率急劇下降，2022 年所進行的第二次田間調查顯示，受 PLV 感染的田地數量明顯減少。沖繩百香果的異常徵狀與 PLV 感染呈現較強的正相關性。

關鍵詞：蚜蟲、修枝剪、RT-PCR、DECS-C、*Carlavirus* 病毒屬。

投稿日期：2025 年 5 月 9 日；接受日期：2025 年 6 月 9 日。

* 通訊作者：ajitomia@pref.okinawa.lg.jp; k-sekine@cs.u-ryukyu.ac.jp
1 Senior Researcher, Okinawa Prefectural Agricultural Research Center, Okinawa, Japan.
2 Graduate Student, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.
3 Research Advisor, Okinawa Prefectural Plant Protection Association, Okinawa, Japan.
4 Associate Professor, Faculty of Agriculture, University of the Ryukyus, Okinawa, Japan.

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):291–301 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0007

Research Article

Comparative Analysis of Xanthine Oxidase and Lipase Inhibition by Extracts from Different *Mesona chinensis* Cultivars

Tzu-Huan Hung[1,*], Chia-Hua Chang[2], Chiu-Hua Chen[2], Tsai-Li Kung[3], and Yang Yang[4]

Abstract

Hung, T. H., C. H. Chang, C. H. Chen, T. L. Kung, and Y. Yang. 2025. Comparative analysis of xanthine oxidase and lipase inhibition by extracts from different *Mesona chinensis* cultivars. J. Taiwan Agric. Res. 74(3):291–301.

This study investigated the differential inhibitory activities of five major Taiwanese *Mesona chinensis* Benth. cultivars ('Taoyuan No. 1', 'Taoyuan No. 2', 'Taoyuan No. 3', 'TARI No. 1', and 'Shuishang') against xanthine oxidase and lipase and explored their correlations with rosmarinic acid and caffeic acid content. The experimental results revealed significant inter-cultivar variations in the inhibitory activities against both enzymes among the different *M. chinensis* cultivars. 'TARI No. 1' exhibited the strongest xanthine oxidase inhibition (68.5%), while 'Taoyuan No. 2' (59.6%) and 'Taoyuan No. 3' (59.2%) showed greater lipase inhibitory effects. A high positive correlation (r = 0.735) was observed between the rosmarinic acid content of *M. chinensis* extracts and xanthine oxidase inhibition, suggesting rosmarinic acid as a potential indicator for this activity. Conversely, caffeic acid content showed a negative correlation (r = -0.807) with lipase inhibition, implying that other constituents in *M. chinensis* may contribute to lipase inhibitory activity. Notably, 'Taoyuan No. 3', a hybrid of 'Taoyuan No. 1' and 'Taoyuan No. 2', displayed enzyme inhibitory activity similar to that of 'Taoyuan No. 2', suggesting a potentially similar genetic expression pattern for the biosynthetic pathways of active components for enzyme inhibitory activity. These findings provide a basis for selecting specific *M. chinensis* cultivars for targeted metabolic regulation applications.

Key words: *Mesona chinensis*, Xanthine oxidase, Lipase, Enzyme inhibition activity, Constituent analysis.

INTRODUCTION

Hyperuricemia and hyperlipidemia are common metabolic disorders globally (Zhu *et al*. 2011; Husain *et al*. 2022; Zhang *et al*. 2022). Hyperuricemia is typically caused by abnormal uric acid metabolism. Excessive uric acid can form crystals in the joints and tissues, leading to gout attacks and increasing the risk of conditions such as kidney disease, fatty liver, and vascular inflammation (Choi & Ford 2007; Billiet *et al*. 2014). On the other hand, hyperlipidemia is mainly characterized by elevated levels of cholesterol and triglycerides in the blood,

Received: March 25, 2025; Accepted: June 16, 2025.
* Corresponding author, e-mail: thhong@tari.gov.tw
1 Assistant Research Fellow, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
2 Project Assistants, Crop Genetic Resources and Biotechnology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
3 Research Fellow, Taoyuan District Agricultural Research and Extension Station, Taoyuan City, Taiwan, ROC.
4 Assistant Research Fellow, Division of Crop Improvement, Tainan District Agricultural Research and Extension Station, Tainan City, Taiwan, ROC.

which can easily lead to atherosclerosis and arterial obstruction. This significantly increases the risk of cardiovascular diseases, subsequently raising the incidence of life-threatening conditions such as stroke and myocardial infarction (Hamaguchi *et al*. 2005; Bonora 2006). In addition, hyperuricemia and hyperlipidemia often co-occur and interact in metabolic disorders, further exacerbating metabolic disturbances and posing a greater threat to cardiovascular health and kidney function.

Xanthine oxidase (EC 1.17.3.2) and lipase (EC 3.1.1.3) play critical roles in the body's uric acid and fat metabolic pathways (Langin 2006; Battelli *et al*. 2018). Xanthine oxidase is a key enzyme in the human body for synthesizing uric acid. It can catalyze the oxidation of hypoxanthine to xanthine, and then catalyze the oxidation of xanthine to produce uric acid (Chen *et al*. 2016). Lipase, secreted by the pancreas, can catalyze the breakdown of dietary fats (mainly triglycerides) into monoglycerides and free fatty acids for absorption by the intestines (Liu *et al*. 2020). Finding inhibitors of these metabolic enzymes is a common way to develop drugs for metabolic syndrome. For example, allopurinol, a strong inhibitor of xanthine oxidase and a purine analog, is commonly used to treat hyperuricemia and gout (Hung *et al*. 2017). Another example is orlistat, a lipase inhibitor containing a β-lactone ring, which reduces dietary fat absorption and thus achieves weight loss (Richardson *et al*. 2008; Liu *et al*. 2020). These drugs play important roles in the treatment of metabolic diseases such as hyperuricemia and obesity, but their side effects cannot be ignored. For example, allopurinol may cause skin rashes and even severe allergic reactions, such as Stevens-Johnson syndrome (Hung *et al*. 2017). Common side effects of orlistat include oily stools and reduced absorption efficiency of fat-soluble vitamins (Liu *et al*. 2020). In contrast, natural extracts may have milder effects, less irritation to the gastrointestinal tract, and reduce the risk of allergies. This makes search for gentler enzyme inhibitors from natural crops essential.

Mesona chinensis Benth., which belongs to the Lamiaceae family, is a crop whose stems and leaves are rich in gelatin and possess a distinctive aroma. In Taiwan, *M. chinensis* is used to produce Mesona tea, a popular beverage in the summer. In the winter, *M. chinensis* is also prepared as a warm, comforting dessert known as grass jelly, making *M. chinensis* a favored crop year-round. Beyond its culinary uses, *M. chinensis* has traditional medicinal applications. Modern scientific research has further demonstrated the potential of *M. chinensis* extracts to regulate blood uric acid (Jhang *et al*. 2016; Chen *et al*. 2022) and blood lipids levels (Huang *et al*. 2016; Xiao *et al*. 2022). *M. chinensis* is abundant in phenolic acids (Yen & Hung 2000; Chusak *et al*. 2014; Huang *et al*. 2021). Previous studies have indicated that rosmarinic acid, a major component of *M. chinensis*, inhibits xanthine oxidase (Chen *et al*. 2022), while another component caffeic acid exhibits lipid-lowering activity (Xiao *et al*. 2022).

In Taiwan, *M. chinensis* is cultivated on approximately 100 ha, primarily across Taoyuan, Hsinchu, Miaoli, Yunlin, and Chiayi. The principal cultivars in Taiwan include 'Taoyuan No. 1', 'Taoyuan No. 2', 'Taoyuan No. 3', 'TARI No. 1', and 'Shuishang' (Fig. 1). 'Taoyuan No. 1', characterized by its high gelation properties, is the dominant cultivar in the Taoyuan, Hsinchu, and Miaoli. 'Taoyuan No. 2', a semi-erect variety, is grown in Yunlin, Chiayi, and Pingtung. 'Taoyuan No. 3' is a hybrid derived from a cross between 'Taoyuan No. 1' and 'Taoyuan No. 2'. 'TARI No. 1' is cultivated primarily in Miaoli and Hsinchu, while 'Shuishang' is the main cultivar in Shuishang, Chiayi. Currently, no studies have directly compared the metabolic enzyme inhibitory activities of these major *M. chinensis* cultivars. This study aims to investigate the xanthine oxidase and lipase inhibitory activities of five prominent Taiwanese *M. chinensis* cultivars. Using rosmarinic acid and caffeic acid as marker compounds, the study will also explore the correlation between enzyme inhibitory activity and the levels of these key components.

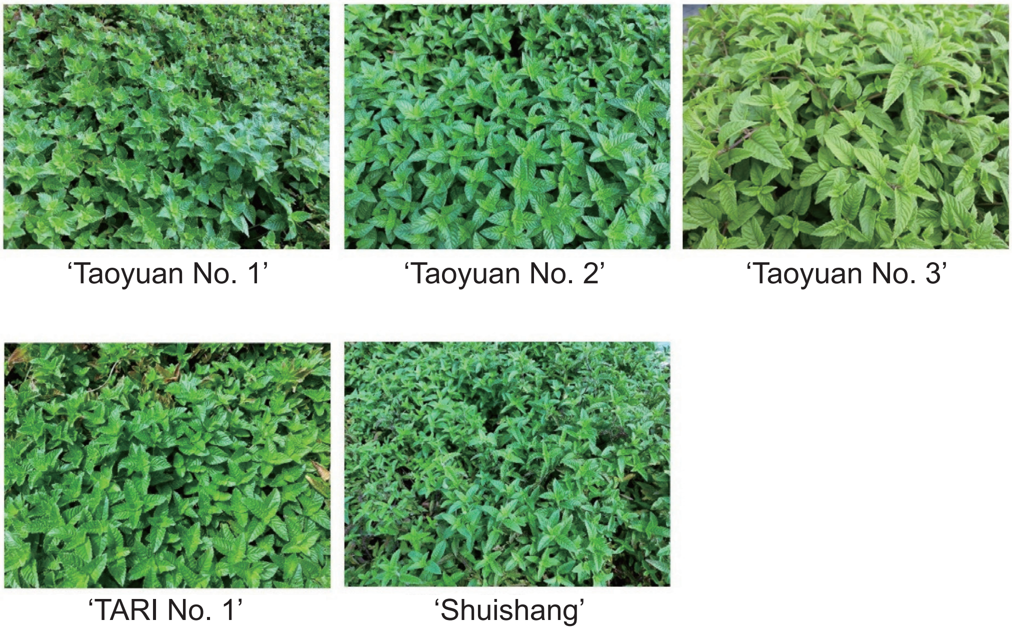

Fig. 1. Five principal *Mesona chinensis* cultivars in Taiwan.

MATERIALS AND METHODS

Plant materials

M. chinensis cultivars 'Taoyuan No. 1', 'Taoyuan No. 2', 'Taoyuan No. 3', and 'TARI No. 1' were collected from the Taoyuan District Agricultural Research and Extension Station, and 'Shuishang' was collected from Shuishang Township, Chiayi County. Stems and leaves were harvested together and sun-dried in the field for 3 d until the moisture content was reduced to below 10%. The dried material was stored at room temperature and remained stable for 6 mo prior to use.

Extraction procedure

The extraction procedure was modified from Jhang *et al*. (2016). Briefly, the dried *M. chinensis* material was ground using a tabletop mill machine (Chyun Tseh, Taichung, Taiwan) and passed through a 20-mesh sieve. The resulting powder was mixed with 50% ethanol at a ratio of 1:20 (w/v). The mixture was subjected to ultrasonic extraction for 1 h at 60℃ with an ultrasonic frequency of 60 Hz. Following extraction, the extract was centrifuged at 14,000× *g*. The supernatant was collected and used for subsequent experiments.

Xanthine oxidase inhibition assay

The xanthine oxidase inhibition assay was modified from Hung *et al*. (2017). Briefly, 100 μL aliquot of the test sample or compound was mixed with 30 μL of xanthine oxidase (X1875, Sigma-Aldrich, Burlington, MA, USA) solution (0.02 U mL^{-1} in 70 mM phosphate buffer, pH 7.5). The mixture was incubated at 25℃ for 15 min. The reaction was initiated by the addition of 70 μL of 150 μM xanthine. The mixture was then incubated in an ELISA reader at 25℃ for 30 min, and the absorbance at 290 nm was measured. The percentage inhibition was calculated according to the following formula: % Inhibition = [1 - (AS at 30 min − AS at 0 min)/(AC at 30 min − AC at 0 min)] × 100, where AS represented the absorbance of mixture which contained all solvents and sample, and AC was the control which contained

all solvent but the sample. The known xanthine oxidase inhibitor, Allopurinol, was used as a positive control. All experiments were performed in triplicate.

Lipase inhibition assay

The lipase inhibition assay was modified from Elbashir *et al*. (2018). Briefly, 50 μL of the test sample was added to 50 μL of lipase (L3126, Sigma-Aldrich, Burlington, MA, USA) solution at a concentration of 90 U mL^{-1} in 200 mM phosphate buffer, pH 7.4. This mixture was incubated at 25℃ for 10 min. Subsequently, 100 μL of 4-methylumbelliferyl oleate (19590, Cayman, Ann Arbor, MI, USA) at a concentration of 0.5 mM was added to the mixture. The mixture was then incubated in a fluorescence ELISA reader at 25℃ for 10 min, and the fluorescence at 460 nm (with an excitation wavelength of 355 nm) was measured. The percentage inhibition was calculated according to the following formula: % Inhibition = [1 - (FS at 10 min − FS at 0 min)/(FC at 10 min − FC at 0 min)] × 100, where FS represents the fluorescence readings of mixture, which contained all solvents and sample, and FC is the control which contained all solvent but the sample. The known lipase inhibitor orlistat was used as a positive control. All experiments were performed in triplicate.

Constituent analysis

Rosmarinic acid and caffeic acid in the *M. chinensis* extracts were quantified using Ultra-Performance Liquid Chromatography (UPLC). The analytical system consisted of an Acquity UPLC H-Class system (Waters Corp., Milford, USA) coupled with a photodiode array detector. The UPLC analysis procedure was modified from Zhang *et al*. (2019). Briefly, samples were filtered through a 0.22 μm syringe filter (Sartorius, Göttingen, Germany) before injection onto an ACQUITY UPLC® HSS T3 column (2.1 × 100 mm; 1.8 μm particle size; Waters, Milford, MA, USA). The column temperature was maintained at 40℃, and the flow rate was set at 0.3 mL min^{-1}. Solvent A consisted of acetonitrile/water/formic acid (2 : 97.9 : 0.1, v/v/v), and Solvent B consisted of acetonitrile/water/formic acid (99.9 : 0 : 0.1, v/v/v). The following gradient elution program was employed: 0 min (92% A and 8% B); 3 min (70% A and 30% B); 7 min (70% A, 30% B); 9 min (0% A, 100% B); 13 min (0% A, 100% B); 14 min (92% A, 8% B); and 15 min (92% A and 8% B). The detection wavelengths were set at 330 nm for rosmarinic acid and 323 nm for caffeic acid, corresponding to their respective wavelengths of maximum absorbance. Calibration curves were generated using a series of standard solutions at varying concentrations. The concentrations of caffeic acid and rosmarinic acid in the extracts were then determined using the respective calibration curves. All samples were measured in triplicate.

Statistical analysis

Differences between groups were analyzed by one-way analysis of variance (ANOVA) using CoStat version 6.400 (CoHort Software, Pacific Grove, CA, USA). If significant differences were detected ($P < 0.05$), Tukey's honestly significant difference (HSD) test was used for post hoc comparisons. Correlation analysis was carried out by SigmaPlot 12.0 (Graffiti LLC, Palo Alto, CA, USA), and significance was determined by a $P < 0.05$.

RESULTS

Quantification of marker compounds

In this study, 50% ethanol extracts from five *M. chinensis* cultivars were subjected to quantitative analysis using UPLC of two marker compounds, caffeic acid and rosmarinic acid (Fig. 2). The results revealed variations in the levels of these marker compounds among the cultivars. For rosmarinic acid content, 'Shuishang' had the highest concentration (80.34 ± 1.65 mg g^{-1}), followed by 'TARI No. 1' (57.76 ± 1.50 mg g^{-1}). 'Taoyuan No. 2' (19.70 ± 0.07 mg g^{-1}) and 'Taoyuan No. 1' (18.37 ± 0.23 mg g^{-1}). 'Taoyuan No. 3' had the lowest rosmarinic acid content (13.20 ± 0.08 mg g^{-1}) (Table 1).

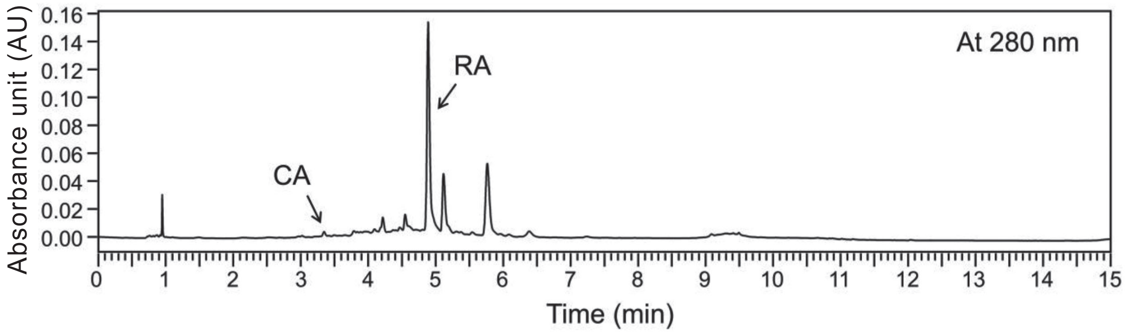

Fig. 2. Ultra-performance liquid chromatography (UPLC) fingerprint at 280 nm of 'Shuishang' extract. RA indicates rosmarinic acid. CA indicates caffeic acid.

Table 1. Analysis of caffeic acid and rosmarinic acid content of extracts from different cultivars of *Mesona chinensis*. Data were expressed as mean ± standard deviation (Mean ± *SD*)

Cultivars	Caffeic acid content (mg g^{-1})	Rosmarinic acid content (mg g^{-1})
'Taoyuan No. 1'	0.13 ± 0.00 bc[z]	18.37 ± 0.23 c
'Taoyuan No. 2'	0.14 ± 0.00 b	19.70 ± 0.07 c
'Taoyuan No. 3'	0.11 ± 0.00 d	13.20 ± 0.08 d
'TARI No. 1'	0.13 ± 0.00 c	57.76 ± 1.50 b
'Shuishang'	0.17 ± 0.01 a	80.34 ± 1.65 a

[z] The difference between means for variables sharing the same letter is not statistically significant (one-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test, $P < 0.05$).

Regarding caffeic acid, 'Shuishang' also exhibited the highest concentration (0.17 ± 0.01 mg g^{-1}), followed by 'Taoyuan No. 2' (0.14 ± 0.00 mg g^{-1}). 'Taoyuan No. 1' (0.13 ± 0.00 mg g^{-1}) and 'TARI No. 1' (0.13 ± 0.00 mg g^{-1}). 'Taoyuan No. 3' had the lowest caffeic acid content (0.11 ± 0.00 mg g^{-1}) (Table 1).

Xanthine oxidase inhibition activity

The inhibitory effects of the 50% ethanol extracts from the five *M. chinensis* cultivars on xanthine oxidase activity were evaluated *in vitro* at a concentration of 100 µg mL^{-1}. The results indicated that 'TARI No. 1' exhibited the highest xanthine oxidase inhibitory activity, significantly greater than the other cultivars, with an inhibition rate of 68.5 ± 1.0%. 'Shuishang' and 'Taoyuan No. 1' followed, with inhibition rates of 61.7 ± 2.1% and 58.9 ± 3.1%, respectively. 'Taoyuan No. 2' and 'Taoyuan No. 3' showed lower inhibition rates, significantly lower than the other cultivars, at 49.6 ± 1.3% and 49.4 ± 2.0%, respectively (Fig. 3). The inhibition rate of the positive control Allopurinol (4 µM) was 91.5 ± 0.4%.

Lipase inhibition activity

The inhibitory effects of 50% ethanol extracts from five *M. chinensis* cultivars on lipase activity were evaluated *in vitro*. The tested samples included extracts from 'Taoyuan No. 1', 'Taoyuan No. 2', 'Taoyuan No. 3', 'TARI No. 1', and 'Shuishang', all at a concentration of 100 µg mL^{-1}. The results revealed that 'Taoyuan No. 2', 'Taoyuan No. 3', and 'Taoyuan No. 1' exhibited higher lipase inhibitory activity, with inhibition rates of 59.6 ± 2.2%, 59.2 ± 4.2%, and 54.1 ± 1.1%, respectively. 'TARI No. 1' showed slightly lower inhibition (50.4 ± 4.7%), but the difference was not statistically significant compared to 'Taoyuan No. 3' and 'Taoyuan No. 1'. 'Shuishang' demonstrated the lowest

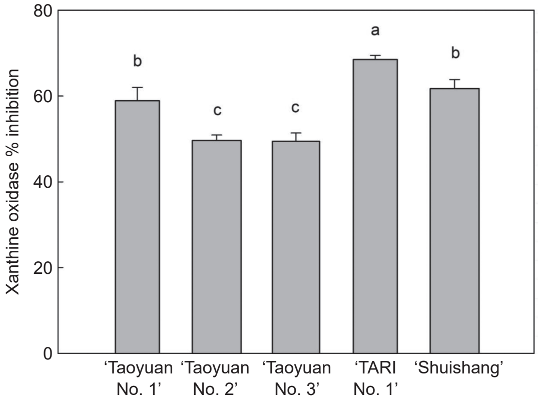

Fig. 3. Analysis of xanthine oxidase inhibitory activity in extracts from five *Mesona chinensis* cultivars ($n = 3$). The difference between means for variables sharing the same letter is not statistically significant (one-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test, $P < 0.05$).

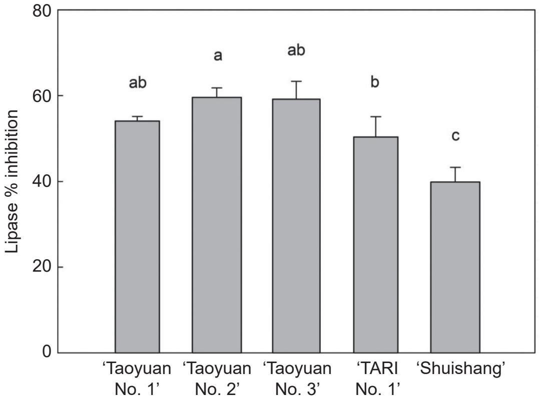

Fig. 4. Analysis of lipase inhibitory activity in extracts from five *Mesona chinensis* cultivars ($n = 3$). The difference between means for variables sharing the same letter is not statistically significant (one-way analysis of variance (ANOVA), Tukey's honestly significant difference (HSD) test, $P < 0.05$).

inhibitory effect, significantly lower than the other cultivars, with an inhibition rate of 39.9 ± 3.4% (Fig. 4). The inhibition rate of the positive control orlistat (20 μM) was 60.2 ± 6.9%.

Correlation analysis

The correlation analysis revealed a highly positive correlation between rosmarinic acid content and xanthine oxidase inhibitory activity in *M. chinensis* extracts, with a correlation coefficient (r) of 0.735 (Fig. 5A). This correlation was statistically significant, as indicated by a P-value of less than 0.05 ($P < 0.05$). Furthermore, a highly negative correlation was observed between caffeic acid content and lipase inhibitory activity, with $r = -0.807$ (Fig. 5B). This negative correlation was also statistically significant ($P < 0.05$).

DISCUSSION

Metabolic regulatory applications of different *M. chinensis* cultivars

While many natural compounds have been reported to possess enzyme inhibitory activities and may serve as potential alternatives to synthetic drugs, their application often faces challenges, such as inconsistent effects due to variations in plant sources (Wu *et al*. 2017; Guo *et al*. 2018; Wang *et al*. 2021). Therefore, systematically evaluating the enzyme inhibitory activities from well-defined plant sources, such as different cultivars, is crucial for identifying more reliable and effective natural metabolic regulators. This study, by comparing the xanthine oxidase and lipase inhibitory activities of five major *M. chinensis* cultivars in Taiwan, aims to provide a more precise basis for developing *M. chinensis*-based metabolic regulatory applications and highlights the differences in activity among cultivars, which can facilitate a more targeted selection and utilization of *M. chinensis* resources for metabolic regulatory applications.

The experimental results demonstrated variations in xanthine oxidase and lipase inhibitory activities among five *M. chinensis* cultivars (Figs. 3 and 4), suggesting the potential to recommend specific cultivars for metabolic regulation applications. In previous studies, 50% ethanol has been identified as the optimal ethanol ratio for extraction solvent to maximize total phenolic content from *M. chinensis* (Jhang *et al*. 2016). Notably, 50% ethanol extracts of *M. chinensis* have also demonstrated favorable xanthine oxidase inhibitory activity,

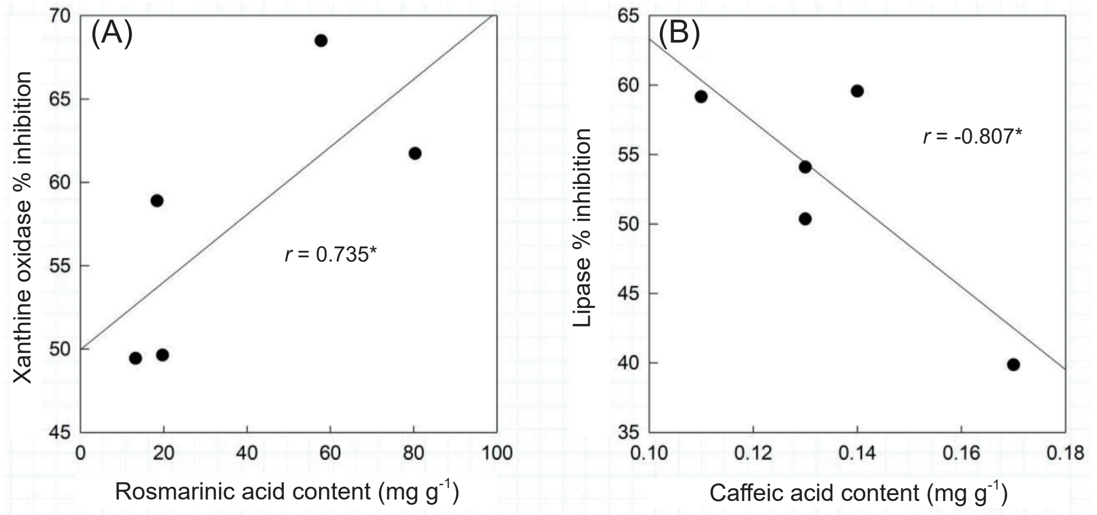

Fig. 5. Relationships in *Mesona chinensis* extracts between (A) rosmarinic acid content and xanthine oxidase inhibitory activity and (B) caffeic acid content and lipase inhibitory activity. The correlation coefficients (r) are annotated on the figure. * indicates significance at $P < 0.05$.

with an IC_{50} of 17.3 ± 2.2 μg mL^{-1} (compared to allopurinol, a positive control, at 1.6 μg mL^{-1}) (Chen *et al*. 2022). Furthermore, when compared to hyperuricemic ICR mice, the 50% ethanol extracts of *M. chinensis* significantly reduced serum uric acid levels in the treatment group (Chen *et al*. 2022), indicating the potential of 50% ethanol extracts of *M. chinensis* for uric acid lowering both *in vitro* and *in vivo*. Therefore, in this comparative study of cultivars in xanthine oxidase inhibition assay, we consistently used 50% ethanol extracts to ensure that variations in observed activities were attributable to cultivar differences rather than extraction efficiency. 'TARI No. 1' exhibited superior performance in xanthine oxidase inhibition (68.5% inhibition rate) (Fig. 3), indicating its potential for uric acid metabolism regulation. Other *M. chinensis* cultivars, such as 'Shuishang' and 'Taoyuan No. 1', although displaying slightly lower xanthine oxidase inhibition activity than 'TARI No. 1', still maintained inhibition rates above 50% (61.7% and 58.9% inhibition rates, respectively) (Fig. 3), suggesting their potential for uric acid modulation as well.

In contrast, the relationship between 50% ethanol extracts of *M. chinensis* extracts and lipase inhibition is still unclear. Although 50% ethanol extracts of *M. chinensis* have been found to ameliorate hyperlipidemia in high-fat diet-induced mice and rats (Yang *et al*. 2024), we found no literature exploring the lipase inhibitory activity of *M. chinensis* extracts. Therefore, this study may be the first to investigate the inhibitory effect of *M. chinensis* extracts on lipase, providing insight into the potential mechanisms by which *M. chinensis* extracts improve hyperlipidemia. Among all *M. chinensis* cultivars, 'Taoyuan No. 2' and 'Taoyuan No. 3' exhibited superior lipase inhibition (59.6% and 59.2% inhibition rates, respectively) (Fig. 4), suggesting their potential for blood lipid control and weight management. 'Taoyuan No. 1' and 'TARI No. 1' showed slightly lower lipase inhibition rates (54.1% and 50.4% inhibition rates, respectively) (Fig. 4), but their inhibition rates remained above 50%, suggesting their potential to also influence blood lipid levels. 'Shuishang' exhibited the lowest lipase inhibition effect (39.9% inhibition rate) (Fig. 4), suggesting it may not be suitable for blood lipid regulation.

Given that *M. chinensis* extracts have been

shown to have hypouricemic and hypolipidemic effects in animal models (Jhang *et al*. 2016; Xiao *et al*. 2022; Yang *et al*. 2024), the observed *in vitro* enzyme inhibitory activities provide mechanistic support for their potential in metabolic regulation. The enzyme inhibitory activities of *M. chinensis* extracts may also indicate their potential *in vivo* efficacy in reducing uric acid or lipid levels. Future studies evaluating the *in vivo* effects of extracts from different *M. chinensis* cultivars in animal models or human subjects are warranted. The inhibitory activities of different *M. chinensis* cultivar extracts against various enzymes show discrepancies. For example, 'Taoyuan No. 2' and 'Taoyuan No. 3' exhibited the lowest xanthine oxidase inhibition activity but the highest lipase inhibition activity (Figs. 3 and 4). Therefore, suitable *M. chinensis* cultivars may vary for different metabolic conditions. Overall, different *M. chinensis* cultivars can be selectively applied to regulate lipid or uric acid metabolism. For complex metabolic syndromes, extracts from different cultivars may be combined to form multifunctional metabolic regulators, achieving more comprehensive health management effects.

Relationship between contents of marker compounds and enzyme inhibitory activity

Previous studies utilizing mass spectrometry have identified rosmarinic acid as a major component from the *M. chinensis* fraction exhibiting high xanthine oxidase inhibitory activity (Chen *et al*. 2022). Rosmarinic acid is a natural polyphenolic compound widely found in Lamiaceae plants, including *M. chinensis*, rosemary (*Rosmarinus officinalis* L.), mint (*Mentha spicata* L.), and perilla (*Perilla frutescens* (L.) Britton) (Takano *et al*. 2004; Shekarchi *et al*. 2012). Rosmarinic acid possesses potent antioxidant capabilities, effectively scavenging free radicals. Moreover, rosmarinic acid exhibits xanthine oxidase inhibitory activity (Chen *et al*. 2022; Tipsuwan *et al*. 2024), suggesting its potential application in preventing and treating hyperuricemia and gout. Our results demonstrated that *M. chinensis* indeed contains abundant rosmarinic acid (13.20–80.34 mg g^{-1}) (Table 1). The variations in rosmarinic acid content among cultivars also indicate potential differences in secondary metabolite biosynthesis and functional activity across these varieties. A highly positive correlation was observed between the rosmarinic acid content and the xanthine oxidase inhibition rate (Fig. 5A). For example, 'Shuishang' (80.3 mg g^{-1}) and 'TARI No. 1' (57.8 mg g^{-1}), had the highest rosmarinic acid contents and also exhibited stronger inhibitory effects on xanthine oxidase, with inhibition rates of 61.7% and 68.5%, respectively (Table 1 and Fig. 3). This supports the potential role of rosmarinic acid in inhibiting xanthine oxidase, consistent with its known anti-hyperuricemic properties.

Caffeic acid is considered as an effective lipase inhibitor (Buchholz & Melzig 2015). However, in our results, a negative correlation between caffeic acid content and lipase inhibitory activity was found in the *M. chinensis* extracts. For instance, 'Shuishang' had the highest caffeic acid content (0.17 mg g^{-1}), but showed the lowest lipase inhibitory effect (39.9%). Conversely, 'Taoyuan No. 2' (0.14 mg g^{-1}) and 'Taoyuan No. 1' (0.13 mg g^{-1}), which had relatively lower caffeic acid contents, performed well in the lipase inhibition assay, with inhibition rates of 54.1% and 59.6%, respectively (Table 1 and Fig. 4). The observed negative correlation between caffeic acid content and lipase inhibitory activity (Fig. 5B) in our *M. chinensis* extracts suggests that under the 50% ethanol extraction conditions, caffeic acid may not be the primary component responsible for lipase inhibition, or its effects might be masked by other compounds present at higher concentrations. This inverse relationship could be attributed to the relatively low concentration of caffeic acid in the 50% ethanol extracts. While 50% ethanol extracts exhibited high total phenolic content (Jhang *et al*. 2016), the concentration of caffeic acid decreases in fractions with higher ethanol concentrations

(Xiao *et al*. 2022). Other compounds in *M. chinensis* related to lipid metabolism, such as kaempferol-7-O-glucoside, rosmarinic acid-3-O-glucoside, and apigenin-3-O-rutinoside (Xiao *et al*. 2022), may also play a role in lipase inhibition. Further investigation is needed to explore these potential candidates.

Enzyme inhibition activity between hybrid cultivar and parental lines

'Taoyuan No. 3', a hybrid cultivar derived from 'Taoyuan No. 1' and 'Taoyuan No. 2', exhibited intermediate lipase and xanthine oxidase inhibitory activities compared to its parent cultivars. Notably, the inhibitory activities against both enzymes of 'Taoyuan No. 3' were closer to those of 'Taoyuan No. 2' (Figs. 3 and 4). This observation aligns with the UPLC fingerprint profiles, which also revealed greater similarity between 'Taoyuan No. 3' and 'Taoyuan No. 2' (data not shown). These findings suggest a potential inheritance pattern where 'Taoyuan No. 3' may be inherited a higher proportion of genetic determinants related to the biosynthesis of the active components from 'Taoyuan No. 2'. Further investigation about the specific components contributing to these differences in enzyme inhibition, as well as the underlying genetic mechanisms, could provide valuable insights for future breeding of *M. chinensis*.

CONCLUSIONS

In summary, rosmarinic acid appears to play a crucial role in the xanthine oxidase inhibitory activity of *M. chinensis* extracts. While caffeic acid has been reported in the literature to possess lipase inhibitory activity, its levels in the *M. chinensis* extracts did not exhibit a significant positive correlation with lipase inhibition. This is likely due to the relatively low caffeic acid content in the *M. chinensis* extracts, suggesting that lipase inhibition activity may depend more on other components. The differences in component composition among the various *M. chinensis* cultivars result in varying potentials for metabolic enzyme inhibition. This provides important information for selecting appropriate *M. chinensis* cultivars for future metabolic regulatory applications.

REFERENCES

Battelli, M. G., M. Bortolotti, L. Polito, and A. Bolognesi. 2018. The role of xanthine oxidoreductase and uric acid in metabolic syndrome. Biochim. Biophys. Acta. Mol. Basis Dis. 1864:2557–2565. doi:10.1016/j.bbadis.2018.05.003

Billiet, L., S. Doaty, J. D. Katz, and M. T. Velasquez. 2014. Review of hyperuricemia as new marker for metabolic syndrome. Intl. Sch. Res. Notices 2014:852954. doi:10.1155/2014/852954

Bonora, E. 2006. The metabolic syndrome and cardiovascular disease. Ann. Med. 38:64–80. doi:10.1080/07853890500401234

Buchholz, T., and M. F. Melzig. 2015. Polyphenolic compounds as pancreatic lipase inhibitors. Planta Med. 81:771–783. doi:10.1055/s-0035-1546173

Chen, C., J. M. Lü, and Q. Yao. 2016. Hyperuricemia-related diseases and xanthine oxidoreductase (XOR) inhibitors: An overview. Med. Sci. Monit. 22:2501–2512. doi:10.12659/MSM.899852

Chen, C. Y., F. C. Hsu, S. F. Tsai, and S. S. Lee. 2022. Bioactive constituents from *Mesona chinensis*: Chemical evidence for its antihyperuricemic activity. J. Chin. Chem. Soc. 69:557–568. doi:10.1002/jccs.202200003

Choi, H. K. and E. S. Ford. 2007. Prevalence of the metabolic syndrome in individuals with hyperuricemia. Amer. J. Med. 120:442–447. doi:10.1016/j.amjmed.2006.06.040

Chusak, C., T. Thilavech, and S. Adisakwattana. 2014. Consumption of *Mesona chinensis* attenuates postprandial glucose and improves antioxidant status induced by a high carbohydrate meal in overweight subjects. Amer. J. Chin. Med. 42:315–336. doi:10.1142/S0192415X14500219

Elbashir, S. M. I., H. P. Devkota, M. Wada, N. Kishimoto, M. Moriuchi, T. Shuto, ... T. Watanabe. 2018. Free radical scavenging, α-glucosidase inhibitory and lipase inhibitory activities of eighteen Sudanese medicinal plants. BMC Complement. Altern. Med. 18:282. doi:10.1186/s12906-018-2346-y

Guo, H., S. Lin, M. Lu, J. D. B. Gong, L. Wang, Q. Zhang, ... D. T. Wu. 2018. Characterization, *in vitro* binding properties, and inhibitory activity on pancreatic lipase of β-glucans from different Qingke (Tibetan hulless barley) cultivars. Intl. J. Biol. Macromol.

120:2517–2522. doi:10.1016/j.ijbiomac.2018.09.023

Hamaguchi, M., T. Kojima, N. Takeda, T. Nakagawa, H. Taniguchi, K. Fujii, ... K. Ida. 2005. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann. Intern. Med. 143:722–728. doi:10.7326/0003-4819-143-10-200511150-00009

Huang, H. C., S. H. Chuang, Y. C. Wu, and P. M. Chao. 2016. Hypolipidaemic function of Hsian-tsao tea (*Mesona procumbens* Hemsl.): Working mechanisms and active components. J. Funct. Foods 26:217–227. doi:10.1016/j.jff.2016.07.019

Huang, J., L. Ding, W. Tian, H. Zhi, J. Chen, L. Wu, ... J. Zheng. 2021. Polyphaenolic profiling, antioxidant properties, and inhibition of α-glucosidase of *Mesona chinensis* benth from Southern China. Microchem. J. 168:106399. doi:10.1016/j.microc.2021.106399

Hung, T. H., J. S. Lai, S. Y. Lin, C. Yang, and Y. L. Lee. 2017. Inhibitory activity of *Alpinia galanga* rhizomes on xanthine oxidase. J. Taiwan Agric. Res. 66:333–342. (in Chinese with English abstract) doi:10.6156/JTAR/2017.06604.08

Husain, M. J., G. Spencer, R. Nugent, D. Kostova, and P. Richter. 2022. The cost-effectiveness of hyperlipidemia medication in low- and middle-income countries: A review. Glob. Heart. 17:18. doi:10.5334/gh.1097

Jhang, J. J., J. W. Ong, C. C. Lu, C. L. Hsu, J. H. Lin, J. W. Liao, and G. C. Yen. 2016. Hypouricemic effects of *Mesona procumbens* Hemsl. through modulating xanthine oxidase activity *in vitro* and *in vivo*. Food Funct. 7:4239–4246. doi:10.1039/C6FO00822D

Langin, D. 2006. Adipose tissue lipolysis as a metabolic pathway to define pharmacological strategies against obesity and the metabolic syndrome. Pharmacol. Res. 53:482–491. doi:10.1016/j.phrs.2006.03.009

Liu, T. T., X. T. Liu, Q. X. Chen, and Y. Shi. 2020. Lipase inhibitors for obesity: A review. Biomed. Pharmacother. 128:110314. doi:10.1016/j.biopha.2020.110314

Richardson, R. D., G. Ma, Y. Oyola, M. Zancanella, L. M. Knowles, P. Cieplak, … J. W. Smith. 2008. Synthesis of novel β-lactone inhibitors of fatty acid synthase. J. Med. Chem. 51:5285–5296. doi:10.1021/jm800321h

Shekarchi, M., H. Hajimehdipoor, S. Saeidnia, A. R. Gohari, and M. P. Hamedani. 2012. Comparative study of rosmarinic acid content in some plants of Labiatae family. Pharmacogn. Mag. 8:37–41. doi:10.4103/0973-1296.93316

Takano, H., N. Osakabe, C. Sanbongi, R. Yanagisawa, K. I. Inoue, A. Yasuda, ... T. Yoshikawa. 2004. Extract of *Perilla frutescens* enriched for rosmarinic acid, a polyphenolic phytochemical, inhibits seasonal allergic rhinoconjunctivitis in humans. Exp. Biol. Med. 229:247–254. doi:10.1177/153537020422900305

Tipsuwan, W., K. Pintha, M. Suttajit, P. Tantipaiboonwong, P. Nuntaboon, and W. Chaiwangyen. 2024. Antioxidant and xanthine oxidase inhibitory activities of rosmarinic acid-rich extract from perilla seed meal. Sci. Asia. 50:2024102. doi:10.2306/scienceasia1513-1874.2024.102

Wang, R., F. Zhang, S. Zan, C. Gao, C. Tian, and X. Meng. 2021. Quality characteristics and inhibitory xanthine oxidase potential of 21 sour cherry (*Prunus cerasus* L.) varieties cultivated in China. Front. Nutr. 8:796294. doi:10.3389/fnut.2021.796294

Wu, Y., Q. Zhou, X. Y. Chen, X. Li, Y. Wang, and J. L. Zhang. 2017. Comparison and screening of bioactive phenolic compounds in different blueberry cultivars: Evaluation of anti-oxidation and α-glucosidase inhibition effect. Food Res Intl. 100:312–324. doi:10.1016/j.foodres.2017.07.004

Xiao, L., X. Lu, H. Yang, C. Lin, L. Li, C. Ni, ... P. Yan. 2022. The antioxidant and hypolipidemic effects of *Mesona Chinensis* Benth extracts. Molecules 27:3423. doi:10.3390/molecules27113423

Yang, H., X. Song, X. Huang, B. Yu, C. Lin, J. Du, ... P. Yan. 2024. *Mesona chinensis* Benth. extract ameliorates hyperlipidemia in high-fat diet-fed mice and rats by regulating the gut microbiota. Foods 13:3383. doi:10.3390/foods13213383

Yen, G. C. and C. Y. Hung. 2000. Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.). Food Res. Intl. 33:487–492. doi:10.1016/S0963-9969(00)00073-9

Zhang, J., T. Chen, K. Li, H. Xu, R. Liang, W. Wang, ... B. Yang. 2019. Screening active ingredients of rosemary based on spectrum-effect relationships between UPLC fingerprint and vasorelaxant activity using three chemometrics. J. Chromatogr. B. 1134–1135:121854. doi:10.1016/j.jchromb.2019.121854

Zhang, Q., H. Li, D. Xing, and J. Lin. 2022. Global research trends in hyperuricemia: A bibliometric and visualized study. Mod. Rheumatol. 32:619–625. doi:10.1093/mr/roab031

Zhu, Y., B. J. Pandya, and H. K. Choi. 2011. Prevalence of gout and hyperuricemia in the US general population: The National Health and Nutrition Examination Survey 2007–2008. Arthritis Rheum. 63:3136–3141. doi:10.1002/art.30520

不同仙草品種萃取物對黃嘌呤氧化酶與脂肪酶抑制活性的比較分析

洪子桓 [1,*] 張家華 [2] 陳秋樺 [2] 龔財立 [3] 楊颺 [4]

摘要

洪子桓、張家華、陳秋樺、龔財立、楊颺。2025。不同仙草品種萃取物對黃嘌呤氧化酶與脂肪酶抑制活性的比較分析。台灣農業研究 74(3):291–301。

本研究旨在探討臺灣 5 種主要仙草 (*Mesona chinensis* Benth.) 品種 (「桃園 1 號」、「桃園 2 號」、「桃園 3 號」、「農試 1 號」及「水上種」) 對黃嘌呤氧化酶 (xanthine oxidase) 與脂肪酶 (lipase) 的抑制活性差異，並探究其與迷迭香酸 (rosmarinic acid) 與咖啡酸 (caffeic acid) 含量的相關性。實驗結果顯示，不同仙草品種間對 2 種酵素的抑制活性存在顯著的品種間差異。其中，「農試 1 號」表現出最強的黃嘌呤氧化酶抑制活性 (68.5%)，而「桃園 2 號」 (59.6%) 與「桃園 3 號」 (59.2%) 則對脂肪酶展現較佳的抑制效果。仙草萃取物中迷迭香酸含量與黃嘌呤氧化酶抑制率呈現高度正相關 (r = 0.735)，顯示迷迭香酸可能適合作為黃嘌呤氧化酶抑制活性的指標成分。相反地，咖啡酸含量與脂肪酶抑制活性呈現負相關 (r = -0.807)，暗示仙草中其他成分可能影響脂肪酶抑制活性。值得注意的是，作為「桃園 1 號」與「桃園 2 號」雜交後代的「桃園 3 號」，其酵素抑制活性與「桃園 2 號」相似，這暗示「桃園 3 號」酵素抑制活性成分的生物合成途徑可能展現與「桃園 2 號」更為接近的遺傳表達模式。本研究結果為未來選擇合適的仙草品種應用於目標代謝調節提供了依據。

關鍵詞：仙草、黃嘌呤氧化酶、脂肪酶、酶抑制活性、成分分析。

投稿日期：2025 年 3 月 25 日；接受日期：2025 年 6 月 16 日。

[*] 通訊作者：thhong@tari.gov.tw
[1] 農業部農業試驗所遺傳資源及生物技術組助理研究員。臺灣 臺中市。
[2] 農業部農業試驗所遺傳資源及生物技術組計畫助理。臺灣 臺中市。
[3] 農業部桃園區農業改良場研究員。臺灣 桃園市。
[4] 農業部臺南區農業改良場作物改良科助理研究員。臺灣 臺南市。

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):303–316 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0008

研究報告

長期施用養豬廢水對水稻生產與土壤性質之影響

吳錫家 [1] 劉嘉仁 [2] 施雅惠 [3] 林旻頡 [3] 廖崇億 [4] 陳琦玲 [5,*]

摘要

吳錫家、劉嘉仁、施雅惠、林旻頡、廖崇億、陳琦玲。2025。長期施用養豬廢水對水稻生產與土壤性質之影響。台灣農業研究 74(3):303–316。

我國自 2011 年起開放申請畜牧廢水農地再利用，但尚未知長期施用下對作物生產與土壤環境的影響。本研究經過 19 年 (2004–2023 年) 長期監測結果，比較施用等量氮、磷、鉀肥分之化肥與養豬廢水對農地與產量之影響，顯示農地長期施灌養豬廢水對土壤 pH 與電導度 (electrical conductivity; EC) 的變化及作物產量沒有顯著差異，但土壤有機質含量會逐漸增加。廢水中銅的主要累積在土壤中，相較試驗前約增加 6 mg kg^{-1}，鋅的累積則在稻稈中。依質量平衡原則，以食用作物農地土壤污染監測標準值之 85% 為上限，計算土地使用年限，顯示若畜牧廢水中氮/銅與氮/鋅之比例超過 180 與 360，則可使土地使用年限超過 100 年以上，但仍建議藉由減少飼料中銅鋅的添加，以延長可施用於農地之年限。經由健康危害商數評估結果顯示，食用經長期施灌養豬廢水造成銅、鋅累積之稻穀，不致對人體造成危害。

關鍵詞：銅、鋅、農地再利用、水稻。

前言

根據農業部 2023 年糧食供需年報統計 (https://agrstat.moa.gov.tw/sdweb/public/book/book.aspx)，全臺豬、牛在養頭數分別為 531.9 萬頭、15.6 萬頭，產生之廢水相當可觀。為達到排放標準，畜牧業者藉由 3 段式處理廢水，其過程既耗能源又損失可用資源，且所費不貲。在歐美國家，長期以來禽畜糞尿被直接作為肥料施用於農田，並未造成公共衛生或環保等問題，農業部農業試驗所 (簡稱農試所) 遂依據廢棄物清理法規，於 2009–2011 年間進行『養豬廢水農地再利用試驗計畫』，評估結果如下：(1) 無論以注入式、噴撒式或隨灌溉水溝灌施肥，其周界異味濃度均在固定污染源空氣污染物排放標準之內，(2) 試驗期間並未發現人畜共通傳染病源或寄生蟲散播問題，(3) 試驗監測期間並未發現地下水受到氮素與銅及鋅污染的現象，(4) 試驗期間土壤特性變化未達顯著差異，但依據銅、鋅質量平衡計算可知畜牧廢水中銅或鋅會在土壤逐漸累積。

基於前述試驗結果，自 2011 年起，農業部依『農業事業廢棄物再利用管理辦法』，開放畜牧廢水再利用個案許可申請，允許在條件管理下多元化再利用畜牧廢水，但若土壤與地下水測值超過食用作物農地土壤監測標準限值之 85%，以及地下水氨氮濃度第二類地下水污染監測標準，則須停止灌溉。環境部亦於 2015 年修訂公告『水污染防治措施及檢測申報管理辦法』第 70 條規定，使畜牧糞尿經厭氧發酵後產生之沼液與沼渣亦可申請施灌於農地，作為農地肥分利用。

投稿日期：2025 年 1 月 9 日；接受日期：2025 年 5 月 26 日。
* 通訊作者：chiling@tari.gov.tw
[1] 農業部農業試驗所農業技術轉譯中心研究員兼主任。臺灣 臺中市。
[2] 農業部農業試驗所農業技術轉譯中心助理研究員。臺灣 臺中市。
[3] 農業部農業試驗所農化組計畫助理。臺灣 臺中市。
[4] 農業部臺中區農業改良場作物環境科助理研究員。臺灣 彰化縣。
[5] 農業部農業試驗所農業化學組研究員。臺灣 臺中市。

然而，施灌畜牧廢水仍存在銅、鋅累積的風險。本研究係基於 19 年 (2004–2023 年) 試驗數據，瞭解連續施灌畜牧廢水後與停灌後對土壤之變化，並根據稻穀中銅、鋅含量，進行健康風險評估，評析是否會對人體健康構成危害。

材料與方法

試驗設計

試驗地點位於農試所 85 號試驗田 (85 號田)，面積 0.5 ha，為砂頁岩與粘板岩混合之非石灰性沖積土，土系為社腳系，質地為壤土。試驗共分成 3 種處理：(1) 不施肥之對照組 (control check; CK)、(2) 施用化肥處理 (chemical fertilizer) 及 (3) 施用糞肥處理 (manure)。水稻品種為「台農 71 號」(*Oryza sativa* ssp. *japonica*., cultivar 'TNG71')，每年進行 2 期作，單期作施肥量為 N：P_2O_5：K_2O = 120：90：85 kg ha^{-1}。

試驗自 2004 年 8 月開始進行至 2023 年 10 月，共 19 年。其中糞肥處理區自 2017 年起改為施用化肥，以觀察停止施用養豬廢水後土壤及水稻之銅、鋅含量變化。

糞肥處理係依據養豬廢水中含氮量，估算單一期作所需氮量需要施用之廢水總量作基肥施用，化肥處理則以『台肥 39 號』複合肥料與硫酸銨施用等效氮量，並於 2 種處理中分別補充過磷酸鈣與氯化鉀，使處理間氮、磷及鉀投入量一致。

於各期作水稻收穫前，每個處理區以逢機採樣方式進行 5 重複點坪割 (10 欉 × 10 欉) 調查產量，每個採樣重複點採集 1 欉水稻與 1 處土壤樣本 (採樣深度：0–15 cm; 15–30 cm) 進行植體元素與土壤特性分析。

畜牧廢水種類與成分分析

國內常見養豬畜舍形式可分成 2 類，分別為平面畜舍與高床畜舍，會因管理方式與冬夏季用水量的不同，導致其廢水中肥分含量差異相當大，因此需藉分析其肥分才能概估施用量。本試驗以高床式養豬畜舍廢水進行施灌，其成分特性如表 1 所示，於每期作施灌前進行分析。分析項目包含酸鹼值 (pH)、電導度 (electrical conductivity; EC)、土壤有機質 (soil organic matter; SOM)、銨態氮 (NH_4^+-N) 與硝酸態氮 (NO_3^--N) 與銅、鋅、鎳、鉻、鎘及鉛含量。分析方法如下：(1) pH：以玻璃電極法測定 (NERA 2019)。(2) EC：以電導度計法測定 (NERA 2023)。(3) SOM：以『豬糞尿灰化分析方法』(Chen *et al*. 2010) 灰化，依灰化後減少量計算 SOM 含量。(4) NH_4^+-N 和 NO_3^--N：以靛酚比色法測定氨氮 (NERA 2020)，以鎘還原法測定硝酸鹽氮 (NERA 2015)。(5) 銅、鋅、鎳、鉻、鎘及鉛：以『豬糞尿灰化分析方法』(Chen *et al*. 2010) 灰化，再以 2N HCl (hydrochloric acid) 洗出灰分與定量後，以感應耦合電漿質譜儀 (Agilent 7500C, Agilent Technologies, Santa Clara, CA, USA) 測定 (NERA 2013)。

土壤與植體分析

土壤樣本風乾後磨碎，並通過 2 mm 篩網，以分析 pH 與 EC；通過 0.5 mm 篩網之土壤樣本，用以分析 SOM、銅、鋅、鎳、鉻、鎘及鉛含量。試驗前表土 (0–15 cm) 分析結果如表 2 所示，分析方法如下：(1) pH：以『土壤 pH 值測定方法–電極法 (S410.62C)』測定 (NERA 2008)。(2) EC：土壤樣品以土水比 1：5 之比例，以電導度計法測定 (Rhoades 1982)。

表 1. 供試高床式畜舍養豬廢水特性。

Table 1. Characteristics of pig wastewater in high-bed type of livestock.

Item	Water content (%)	pH	EC[z] (dS m^{-1})	OM[z] (%)	N (%)	P (%)	K (%)
Average	96	7.8	25	3.5	0.40	0.12	0.22
Range	91–99	7.4–8.3	10–36	1.1–8.3	0.22–0.87	0.01–0.22	0.09–0.35

表 1. 供試高床式畜舍養豬廢水特性。(續)

Table 1. Characteristics of pig wastewater in high-bed type of livestock. (continued)

Item	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cr (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})	As (mg kg^{-1})
Average	24.30	63.20	0.55	0.39	0.02	0.14	0.16
Range	2.08–100.00	4.13–212.00	0.15–1.10	0.07–1.00	0.01–0.08	0.01–1.20	0.01–0.80

[z] EC: electrical conductivity; OM: organic matter.

表 2. 試驗前表土 (0–15 cm) 基本特性。

Table 2. Basic characteristics of initial topsoil (0–15 cm).

Soil series	pH	EC[z] (μS cm^{-1})	OM[z] (%)	Texture	Cu (mg kg^{-1})	Zn (mg kg^{-1})	Ni (mg kg^{-1})	Cr (mg kg^{-1})	Cd (mg kg^{-1})	Pb (mg kg^{-1})
Shejiao series	5.4	59	2.03	Loam Sand (%) = 45 Silt (%) = 38 Clay (%) = 16	15.3	86.3	24.3	35.1	0.21	29.4

[z] EC: electrical conductivity; OM: organic matter.

(3) SOM：『土壤有機質測定方法－燃燒/紅外線測定法 (TARI S201.1B)』(TARI 2013)。

(4) 銅、鋅、鎳、鉻、鎘及鉛含量：『土壤重金屬檢測方法－微波輔助王水消化法』(NERA 2018) 消化後，以感應耦合電漿質譜儀 (Ultima 2C, Horiba Jobin Yvon, Irvine, CA, USA) 測定 (NERA 2013)。

水稻於收穫後分為稻穀與稻稈，分別秤量濕種，並於 60℃烘乾後分別磨碎、過篩 (2 mm)，分析植體元素，包含銅、鋅、鎳、鉻、鎘及鉛含量，分析方法參考『植體巨量元素與微量元素測定－灰化法』，植物樣品經高溫灼燒，除盡水分與 SOM 後，以稀鹽酸加熱溶解、過濾與稀釋後，以感應耦合電漿質譜儀 (Agilent 7500C, Agilent Technologies, Santa Clara, CA, USA) 測定 (NERA 2013)。

健康危害商數 (hazard quotient; HQ)

本研究使用非致癌風險進行計算 HQ，其計算方法如下：

$$HQ = DIM/RFD$$

DIM (daily intake of metals; DIM)：每日金屬攝取量；

RFD (oral reference dose; RFD)：食入參考劑量。

$$DIM = C_{metal} \times C_{factor} \times D_{food\ intake}/B_{average\ weight}$$

C_{metal}：表示稻穀金屬濃度；

C_{factor}：表示溼穀至乾穀重量比例，0.85；

$D_{food\ intake}$：表示稻穀攝取量，依據農業部糧食供需年報，臺灣每人每年食米量為 45.6 kg (https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx)；

$B_{average\ weight}$：表示食用人體重，依據衛福部統計處報告全臺 19–64 歲平均體重為 64 kg (政府資料開放平臺，https://data.gov.tw/dataset/8492)；

RFD：依據美國環境保護署規範各金屬食入參考劑量 (US-EPA IRIS 2006)。

結果與討論

畜牧廢水於不同畜舍及不同季節肥分變化

收集臺中、雲林、臺南共 21 家平面式養豬畜舍經固液分離後之廢水，pH 值範圍介於 6.4–8.8，經分析氮含量平均為 0.11 (0.06–0.24)%，銅含量平均為 2.44 (0.44–5.53) mg kg^{-1}，鋅含量平均為 15.1 (0.8–43.5) mg kg^{-1}。而本研究使用之高床式養豬畜舍經固液分離後之廢水 pH 值範圍介於 7.4–8.3，為中性至微鹼性的溶液性質，廢水氮含量平均為 0.40 (0.22–0.97)%，

銅含量平均為 24.3 (1.11–34.6) mg kg^{-1}，鋅含量平均為 63.2 (4.10–82.8) mg kg^{-1}。相較於平面式養豬廢水，氮濃度相對較高，且變異大；鉀濃度相對較低，而變異小，故氮/鉀比例高，若農地施用高床式養豬廢水以栽培鉀肥需求較高的作物，易有鉀肥不足之問題，因此需額外補充其他鉀肥。以雖然氮與磷平均濃度分別為 0.40 與 0.13%，但養豬廢水中氮與磷濃度會隨著季節有明顯的變化 (圖 1)，可以看出冬季之氮與磷濃度高，氮濃度可高達約 0.59%，冬季平均值約為 0.50%，其他季節平均值約為 0.32%；磷最高濃度約為 0.25%，冬季平均值約為 0.15%，其他季節約為 0.10%；鉀則沒有隨著季節而有明顯的變化，濃度平均大約為 0.22%。

不同處理對水稻產量之影響

藉由坪割後稻穀乾重計算 3 種處理各年度各期作產量比值 (yield ratio)，結果顯示糞肥處理相較於化肥處理彼此間產量無顯著差異 (圖 2)，平均比值為 97.1，糞肥處理較化肥處理之水稻產量減少約 2.5%；而化肥處理與糞肥處理相較於對照組，平均比值分別為 200 與 195。

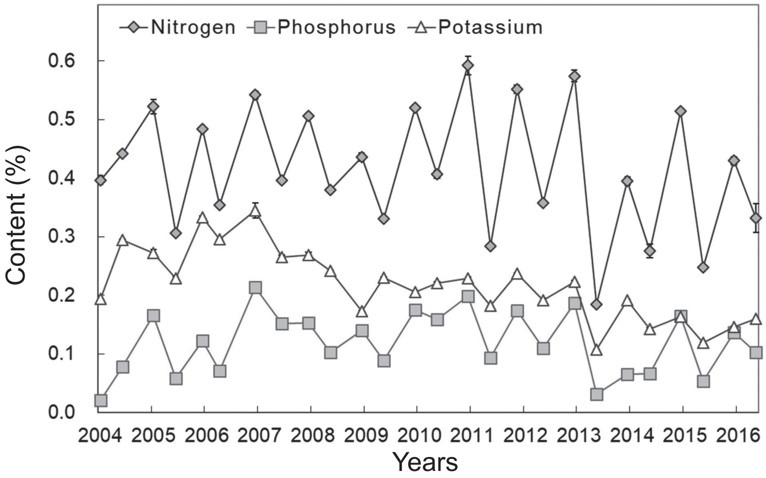

圖 1. 高床式畜舍養豬廢水氮、磷、鉀濃度季節變化。

Fig. 1. Seasonal changes of nitrogen, phosphorus and potassium concentrations in pig wastewater in high-bed type of livestock.

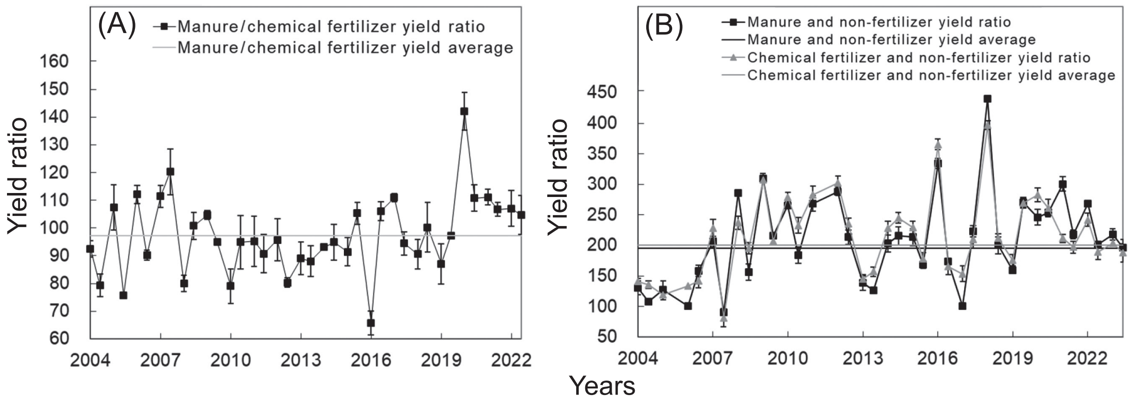

圖 2. 各年度水稻施用糞肥、化肥與不施肥處理間產量比值 (A) 糞肥/化肥產量比值、(B) 糞肥/對照與化肥/對照產量比值。

Fig. 2. The ratio of the rice yield between manure, chemical fertilizer and non-fertilizer in each year: (A) manure/chemical fertilizer yield ratio; (B) manure and chemical fertilizer/non-fertilizer yield ratio.

不同施肥處理長期施用對土壤特性變化

經過長期施用後，不論在表土 (0–15 cm) 或底土 (15–30 cm)，糞肥處理之土壤 pH 值均會提高 (圖 3)，此結果與 Rosa *et al*. (2017) 研究結果相似；化肥處理長期施用後，不論表土或底土，其 pH 值均有略微上升之趨勢；對照組處理於試驗期間，不論表土或底土，其 pH 值均大幅提高。三種不同處理均導致土壤 pH 上升主要可能與灌溉水淹灌水稻有關，圖 4 為農試所灌溉水不同時期之 pH 值，其平均值為 7.9 (6.0–9.1)，雖然長期施用化肥會造成土壤酸化、施用畜牧廢水提高之 SOM 分解後產生之有機酸等均會導致 pH 下降，但在長期高 pH 值之灌溉水淹灌下，pH 值會逐漸增加。畜牧廢水平均 pH 值雖高於原來土壤 pH 值，但糞肥處理區與化肥處理區之土壤 pH 值並無顯著差異，反而對照區的土壤 pH 值明顯高於兩個處理區，這可能與對照區之作物生長趨勢與產量遠低於處理區，導致田區鹽分未被植物吸收而使土壤 pH 與 EC 值高於處理區。而各區在經過 3–4 年試驗處理管理後，該管理系統已達平衡。

依據研究指出，EC 大於 0.85 dS m^{-1}，則對大部分的作物生長造成影響 (中興大學機構典藏，https://ir.lib.nchu.edu.tw/handle/11455/84145)。本試驗進行至今 (2023 年)，結果顯示 3 種不同處理之表土 EC 值最高不超過 0.25 dS m^{-1}、底土最高不超過 0.15 dS m^{-1}，表示三種處理之水稻均未受到鹽害影響，且施用養豬廢水的 EC 值甚至低於施用化肥，其結果與先期研究 (Chen *et al*. 2010) 相似，顯示土壤合理施用養豬廢水後，並無明顯鹽分累積情形。

三種不同處理之SOM含量呈現不同變化，施用化肥處理與糞肥處理之 SOM 含量逐漸上升，分別為 0.14 與 0.6%，而對照組處理之 SOM 含量則逐漸下降，兩者間之差異主要與水稻產量與作物殘體有關，試驗期間每期作整地時會犁入上期作殘留之植物殘體，並經微生物的作用分解與土壤混合後增加 SOM，而水稻殘體與水稻產量占比約為 1 : 1，因此在水稻產量對照組 < 施用畜牧廢水處理 ≒ 施用化肥處理之條件下，對照組之 SOM 含量 < 施用畜牧廢水處理與施用化肥處理 SOM 含量。另外畜牧廢水本身富含豐富之有機物質，因此相較於化肥施用，能提供土壤更多之有機物質而提高 SOM 含量，此結果與 Chen *et al*. (2004) 及 Lucas *et al*. (2013) 長期施灌畜牧廢水會增加 SOM 含量相符，雖然各試驗之施用量不同，但施用含 SOM 之畜牧糞尿水都會增加不同程度之 SOM 含量。

本試驗長期施用養豬廢水相對於試驗前，土壤 pH、EC 及 SOM 均有提高之現象，但提高幅度不高。由此可知長期施灌養豬廢水對土壤 pH 與 EC 的變化影響不大，但持續增加的 SOM 有助於提高土壤肥力與改善土壤物理性質。

不同處理對土壤與植體銅、鋅累積影響

<u>施用糞肥處理</u>

養豬業者為了提高飼料效率與豬隻健康，常在飼料添加銅、鋅 (Takada 2005; Suzuki *et al*. 2010)。添加銅為促進生長，且可作為腸道抗菌劑的低成本的方法 (Rosen & Roberts 1996)，剛斷奶的小豬以高鋅含量食物餵養，可減輕腹瀉情形 (Holm 1990; Hill *et al*. 2000; Hu *et al*. 2013)。銅與鋅是參與代謝反應的兩種微量元素，且是動物體中許多酵素與分子組成之重要因子 (Karoline & Hanne 2017)。然而，動物對飼料中銅鋅卻只能吸收 10–20%，大約 80–90% 的銅鋅皆被排泄至糞尿中 (Takada 2005; Suzuki *et al*. 2010)。因此長期施用養豬廢水容易造成土壤銅、鋅累積 (Aldrich *et al*. 2002; Lucas *et al*. 2013)。

試驗結果中發現廢水中銅主要累積在表土，而鋅、鎳、鉻、鎘及鉛主要累積在稻稈 (圖 5 至圖 7)，故糞肥處理中土壤銅的含量隨著每年廢水的施灌而有逐漸上升之趨勢。自 2004 年施用養豬廢水至 2016 年間，土壤銅含量與對照區、化肥處理區具有顯著差異，相較試驗前約增加 6 mg kg^{-1}；土壤鋅含量與化肥處理區具有顯著差異，相較試驗前約減少 2 mg kg^{-1}；土壤鎳含量各組間皆有顯著差異，相較試驗前約增加 0.24 mg kg^{-1}。停灌養豬廢水後改以化肥施用 (2017–2023 年)，施用糞肥處理區土壤銅含量約下降 0.3 mg kg^{-1}，鋅含量下降 5 mg kg^{-1}，

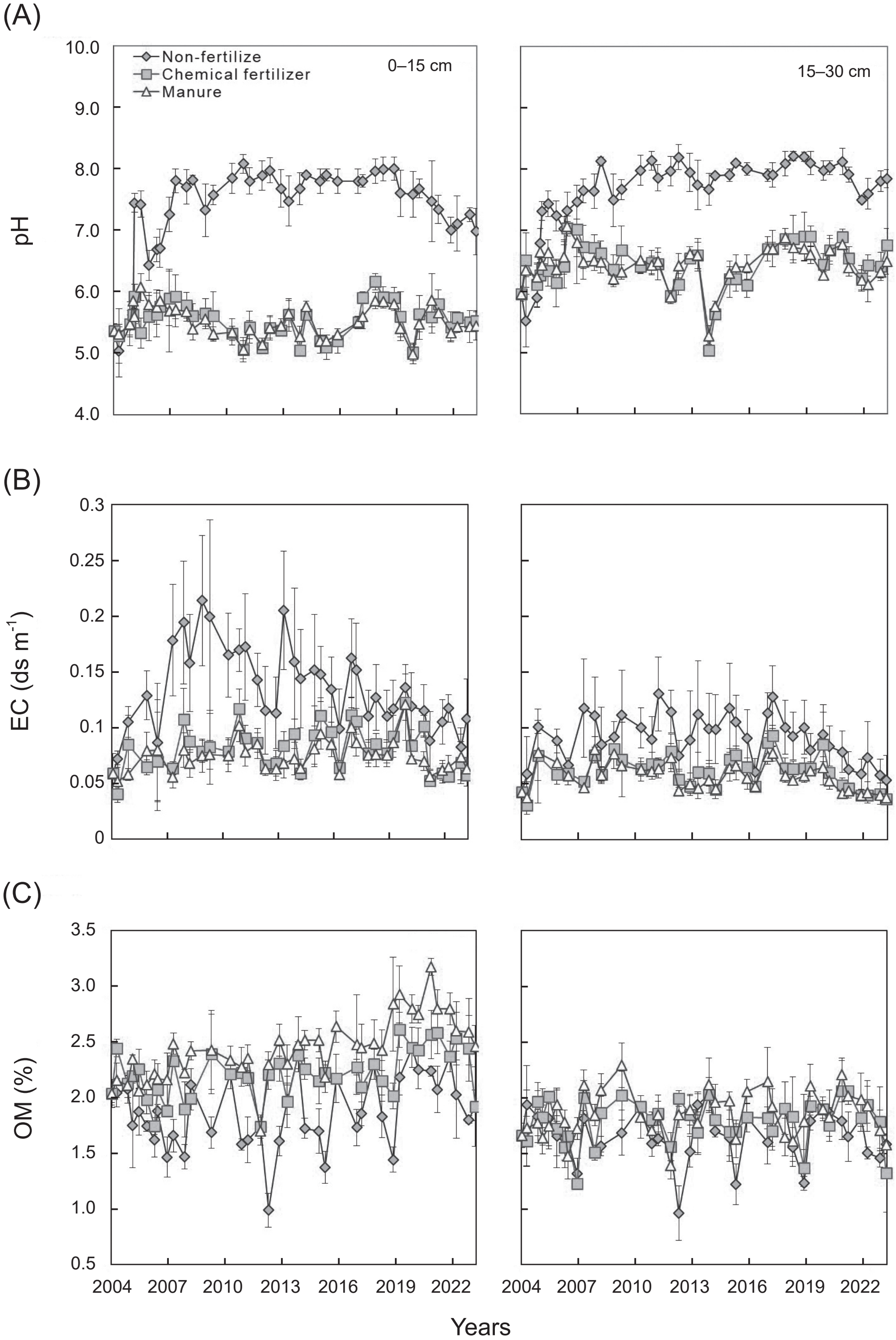

圖3. 施用糞肥、化肥與不施肥處理土壤於表土 (0–15 cm) 與底土 (15–30 cm) (A) pH、(B) EC及 (C) OM變化。

Fig. 3. The changes of (A) pH; (B) electrical conductivity (EC); and (C) organic matter (OM) by application of manure, chemical fertilizer and non-fertilizer treatment in topsoil and subsoil.

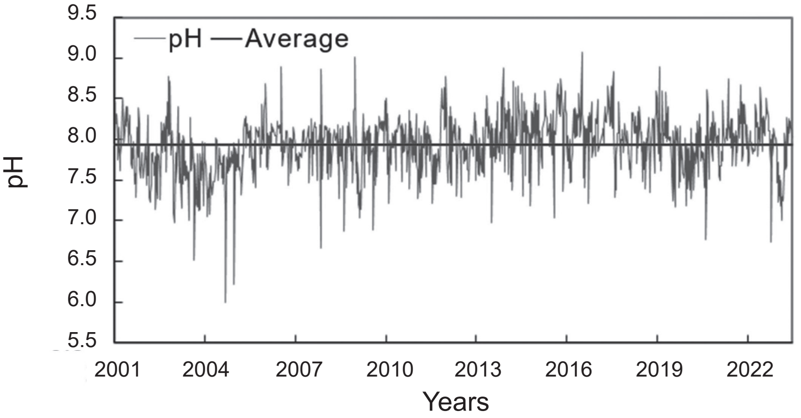

圖 4. 歷年農試所灌溉水 pH 值。

Fig. 4. The pH value of irrigation water in Taiwan Agricultural Research Institute (TARI) in each year.

土壤鎳含量相較試驗前約減少 1.28 mg kg^{-1}，顯示改以化肥施用可降低因施用畜牧廢水造成之重金屬在土壤中的累積。因此，長期施用廢水若土壤銅、鋅達到限值時，即食用作物農地土壤監測標準限值之 85% 時，應停止施灌，預期藉由自然土壤淋洗以及作物殘體移除，土壤銅、鋅含量會逐年下限。然停灌養豬廢水 7 年期間，土壤銅、鋅、鎳及化肥處理區仍具顯著差異，表示地力尚未恢復。

水稻重金屬含量與土壤中重金屬含量之比值

水稻 (稻穀與稻稈) 重金屬含量與其土壤中相對應重金屬含量的比值，可用以評估水稻自土壤中吸收重金屬的能力。比值越高，代表水稻對該重金屬的吸收能力越強。由表 3 結果可知，水稻對鎘的吸收能力最高，其中施用糞肥處理組的鎘比值達 1.99；其次為鋅，其比值為 0.53；而吸收能力最低的為鉛，其比值僅為 0.02。此結果顯示水稻對鎘與鋅具有較高的吸收潛勢，而鉛則較不易被吸收。上述觀察與 Kabata-Pendias & Pendias (1992) 及 Yeh (2002) 的研究結果一致。

長期施用畜牧廢水對土壤使用年限計算

依據質量平衡原則，可估算銅、鋅、鎳、鉻、鎘、鉛、砷在土壤中的累積。以本研究為例，養豬廢水氮含量為 0.4% (表 1)，若以環境部土壤氮處理上限 (400 kg N ha^{-1} yr^{-1})，則每年可施用最大量為 100 Mg ha^{-1} yr^{-1}，即每年銅、鋅、鎳、鉻、鎘、鉛及砷投入量為 2.4、6.3、0.06、0.04、0.002、0.01 及 0.02 kg ha^{-1} yr^{-1}。以表土重量 2,000 Mg ha^{-1} 計算，則每年銅、鋅、鎳、鉻、鎘、鉛及砷增加量為 1.2、3.0、0.0137、0.0045、0.0004、0.0064 及 0.0078 mg kg^{-1}，將施用造成的重金屬投入量扣除移除量 (以稻穀中銅、鋅、鎳、鉻、鎘、鉛及砷含量與收穫量計算) 與土壤銅、鋅、鎳、鉻、鎘、鉛及砷背景含量 (15、86、24、35、0.21、29 及 8.23 mg kg^{-1}) 計算，以土壤停灌基準之 85% 為限值 (102、221、111、149、2.1、255 及 25.5 mg kg^{-1})，土壤可使用年限為 77 (銅)、45 (鋅)、6,350 (鎳)、25,333 (鉻)、4,725 (鎘)、35,313 (鉛) 及 2,208 (砷) (表 4)，從表 4 中可知，銅與鋅為影響農地再利用之重要因子，因此以畜牧廢水施灌農地，需要監測土壤銅與鋅的累積。另經由上述案例計算發現，氮/銅與氮/鋅之比例需超過 180 與 360，可使土地銅使用年限超過 100 年以上，鋅使用年限超過 300 年以上，建議調降飼料中銅鋅的添加以增進土地使用年限。

依質量平衡概估，廢水中銅、鋅平均含量 23.9、63.1 mg L^{-1}，每年土壤銅、鋅增加量分別約為 0.94、2.2 mg kg^{-1}，預期 13 年之土壤銅、鋅累積量分別約為 12.2、29 mg kg^{-1}，但實際施用後，土壤銅、鋅含量實際量測值小於預估值，此為試驗土壤濃度分布不均與每年

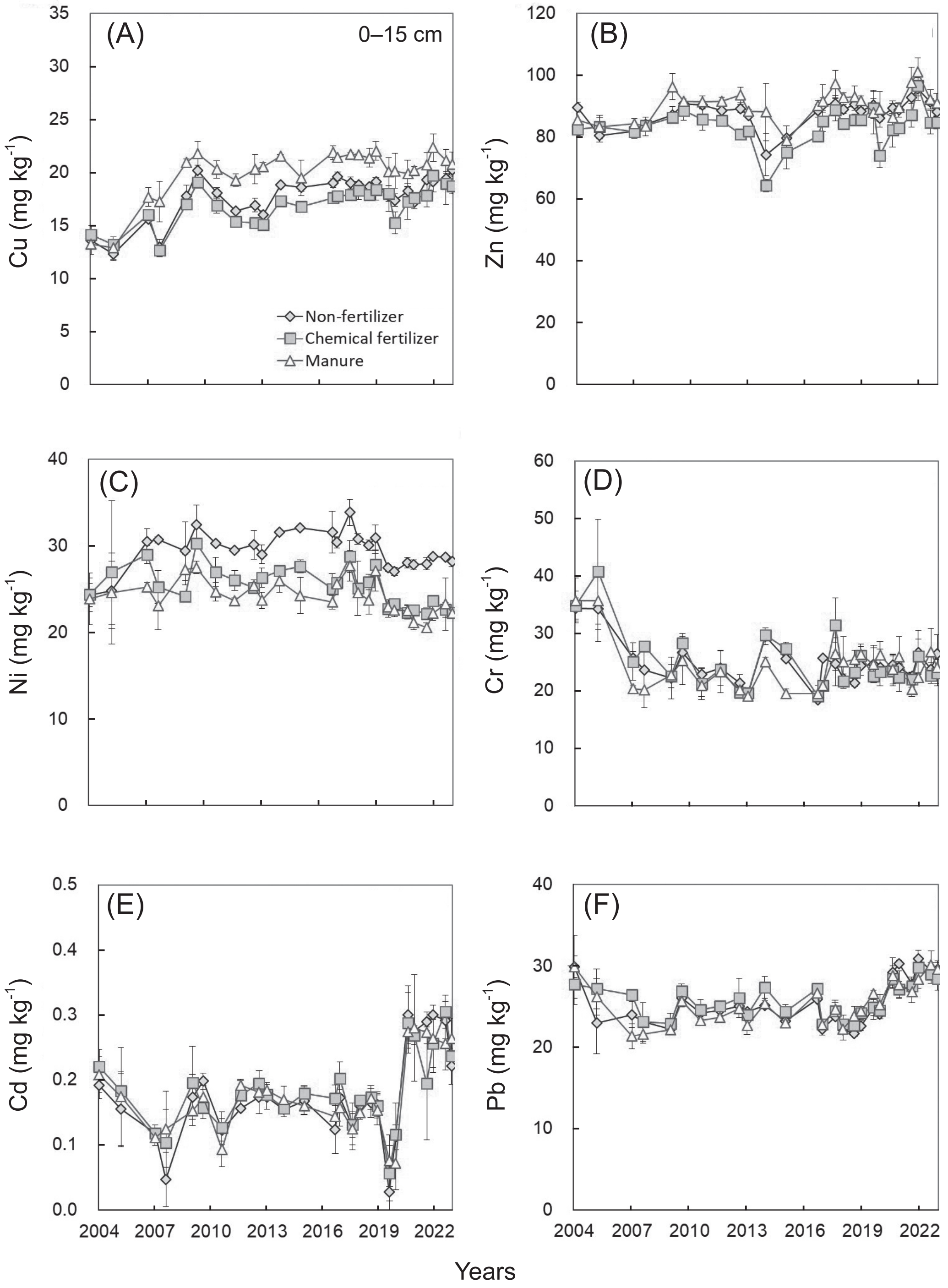

圖 5. 施用糞肥、化肥及不施肥處理之表土 (0–15 cm) 重金屬含量變化 (A) 銅、(B) 鋅、(C) 鎳、(D) 鉻、(E) 鎘及 (F) 鉛。

Fig. 5. The changes of (A) Cu; (B) Zn; (C) Ni; (D) Cr; (E) Cd; and (F) Pb by application of manure, chemical fertilizer and non-fertilizer treatment in topsoil.

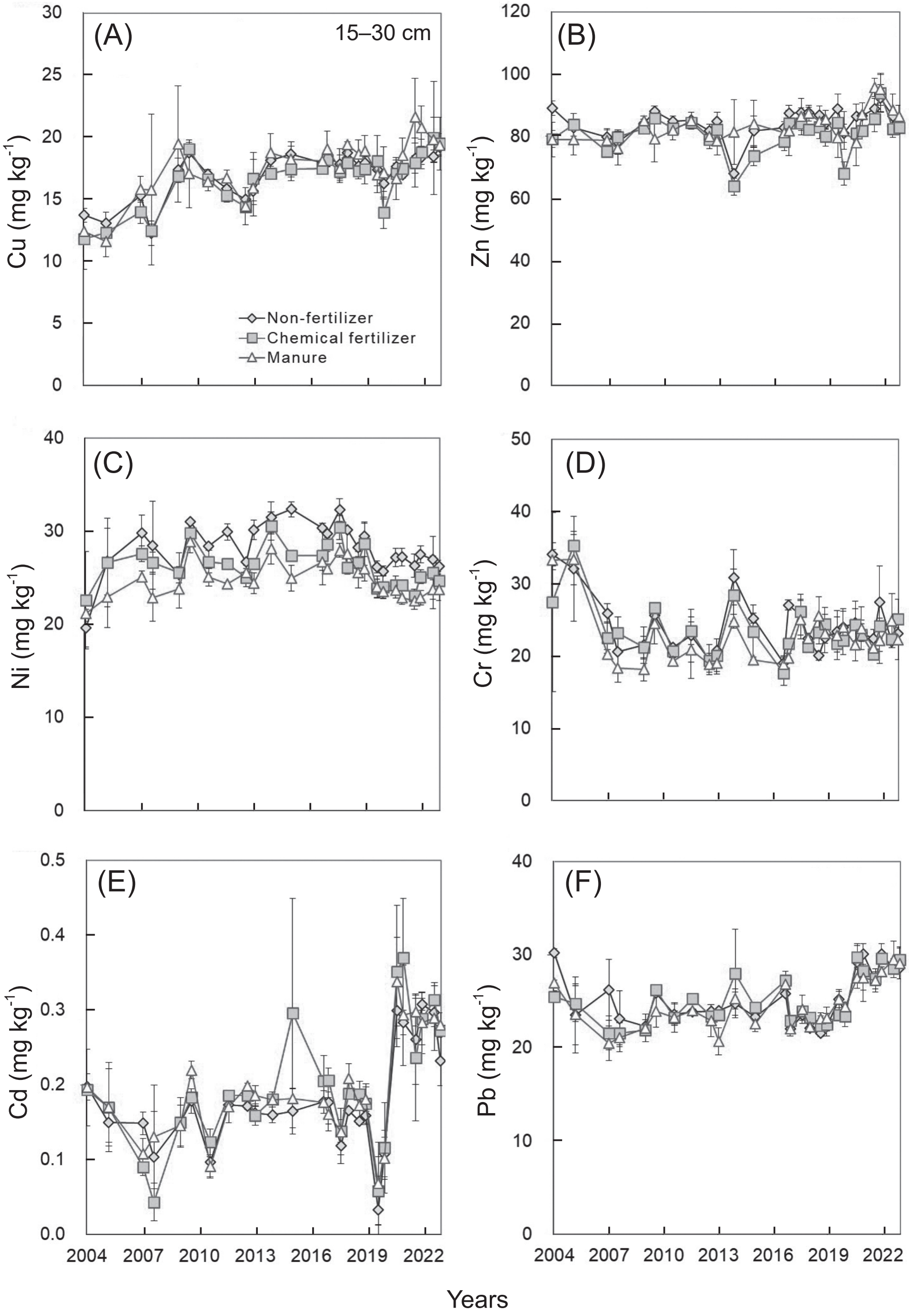

圖 6. 施用糞肥、化肥及不施肥處理之底土 (15–30cm) 重金屬含量變化 (A) 銅、(B) 鋅、(C) 鎳、(D) 鉻、(E) 鎘及 (F) 鉛。

Fig. 6. The changes of (A) Cu; (B) Zn; (C) Ni; (D) Cr; (E) Cd; and (F) Pb by application of manure, chemical fertilizer and non-fertilizer treatment in subsoil.

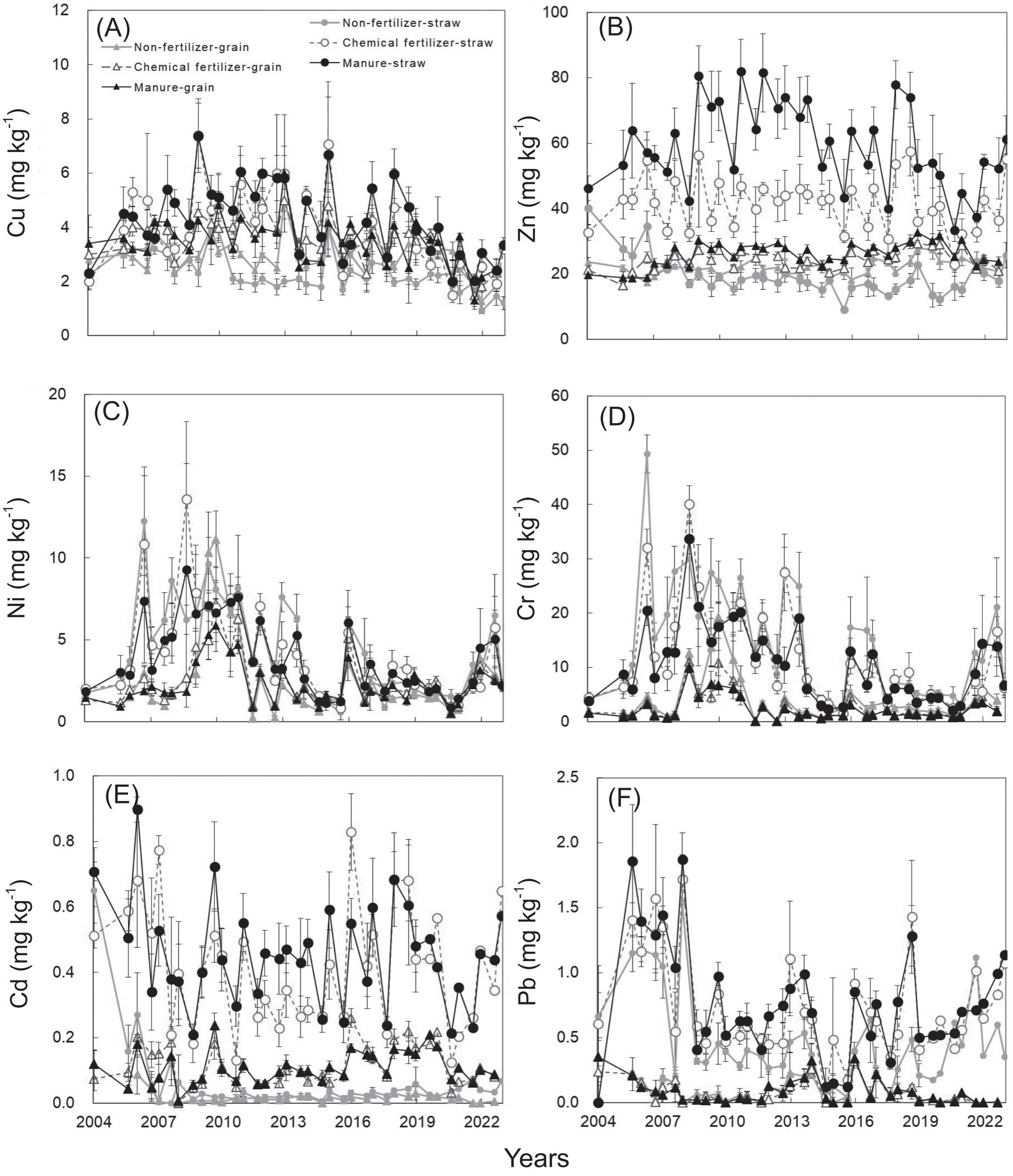

圖 7. 施用糞肥、化肥及不施肥處理之水稻植體重金屬含量變化 (A) 銅、(B) 鋅、(C) 鎳、(D) 鉻、(E) 鎘及 (F) 鉛。

Fig. 7. The changes of (A) Cu; (B) Zn; (C) Ni; (D) Cr; (E) Cd; and (F) Pb by application of manure, chemical fertilizer and non-fertilizer treatment in paddy rice.

表 3. 水稻植體重金屬與土壤重金屬含量之比值。

Table 3. The ratio of paddy rice and soil in heavy metals content.

Item	Cu	Zn	Ni	Cr	Cd	Pb
Paddy rice/soil- Non fertilizer	0.19	0.25	0.13	0.39	0.42	0.02
Paddy rice/soil- Chemical fertilizer	0.29	0.40	0.13	0.32	1.68	0.02
Paddy rice/soil- Manure	0.26	0.53	0.13	0.30	1.99	0.02

表 4. 養豬廢水農地再利用可使用年限估算。

Table 4. Estimated limits of different heavy metals for farmland reuse with pig wastewater.

Metals	Concentration (mg L^{-1})	Dosage[z] (Mg ha^{-1} yr^{-1})	Applied metal dosage (kg ha^{-1} yr^{-1})	Removal amount (kg ha^{-1} yr^{-1})	Increasing amount[y] (mg L^{-1})	Content in soil (mg L^{-1})	Monitoring criteria[x] (mg L^{-1})	The useful life (Years) (yr)
Pig wastewater in high-bed type of livestock (N = 0.4%)								
Cu	24	100	2.40	0.048	1.2	15	102	77
Zn	63	100	6.30	0.3	3.0	86	221	45
Ni	0.55	100	0.06	0.0276	0.0137	24	111	6,350
Cr	0.39	100	0.04	0.03	0.0045	35	149	25,333
Cd	0.02	100	0.002	0.0012	0.0004	0.21	2.1	4,725
Pb	0.14	100	0.01	0.0012	0.0064	29	255	35,313
As	0.16	100	0.02	0.00036	0.0078	8.23	25.5	2,208

[z] Calculated based on the soil treatment upper limit set by the Environmental Protection Agency (EPA) (400 kg ha^{-1} yr^{-1}).

[y] The average concentrations of copper, zinc, nickel, chromium, cadmium, lead, and arsenic in rice grains are 4, 25, 2.3, 2.5, 0.1, 0.1, and 0.03 mg ha^{-1}, respectively, calculating the crop uptake based on a yield of 6,000 kg ha^{-1} per cropping season.

[x] 85% of the limit value for heavy metal monitoring standards in edible crop soils.

施用廢水中之銅與鋅含量不同導致，在未考量植體吸收之銅鋅，每年土壤增加之銅鋅速率與 Novak *et al*. (2004) 結果相似。

健康危害商數 (HQ)

土壤中所含有毒重金屬可藉由食用受污染之食物、水或粉塵進入人體中 (Cambra *et al*. 1999)，在受污染土地上種植的蔬菜可能會累積有毒金屬，長時間食用受污染的食物可能導致人類肝臟和腎臟中有毒金屬的不斷積累，從而導致生化過程受到干擾，例如肝臟，腎臟，心血管，神經及骨骼疾病等 (WHO 1992; Järup 2003)。據估算，超過 70% 以上之鎘攝入量是通過食物鏈提供的 (Wagner 1993)。本研究為瞭解試驗生產之稻穀銅、鋅含量經食用後是否對人體有害，因此以 HQ 進行評估。

因我國僅規範食米中鎘、汞及鉛含量 (HPA, MOHW 2018)，並未規範食入參考劑量，因此依據美國環境保護署規範銅、鋅、鎳、鉻、鎘及鉛食入參考劑量分別為 0.04、0.3、0.02、1.5、0.001 及 0.004 mg kg bw^{-1} d^{-1} (US-EPA IRIS 2006) 進行計算。2018 年臺灣每人每年食米量為 45.6 kg (農業統計年報，https://agrstat.moa.gov.tw/sdweb/public/book/Book.aspx)，以稻穀製成白米約損失 30% 計算，每人每日稻穀食用量為 0.18 kg d^{-1}。依據衛福部統計處臺灣各地區層 19–64 歲成人身高、體重及身體質量指數 (body mass index; BMI) 平均值，全臺 19–64 歲平均體重為 64 kg。經計算 3 處理中銅、鋅、鎳、鉻、鎘及鉛最大濃度攝取量分別為 0.009、0.06、0.007、0.01、0.0003 及 0.0003 mg kg bw^{-1} d^{-1}；銅、鋅、鎳、鉻、鎘及鉛 HQ 分別為 0.225、0.2、0.35、0.007、0.3 及 0.075，所有重金屬 HQ 均小於 1，表示稻穀中之銅、鋅含量對人體影響程度低。

結論

從長期監測數據顯示，農地長期施灌養豬廢水對土壤 pH 與 EC 的變化影響不大，但持續增加的 SOM 有助於提高土壤肥力與改善土壤物理性質。廢水中銅的主要累積在土壤中，鋅的主要累積在稻稈中；累積於稻稈之鋅，隨收穫後稻稈回田，再被下期水稻吸收，建議可移除水稻殘體，避免土壤中鋅累積。由植體重金屬與土壤重金屬含量之比值可知鎘與鋅容易從土壤中被水稻吸收，因此需注意作物鎘與鋅之累積。依質量平衡原則計算土地使用年限可知氮/銅與氮/鋅之比例需超過 180 與 360，可使土地使用年限超過 100 年以上。另建議減少飼料中銅鋅的添加，以延長可施用於農地之年限。經由 HQ 評估結果顯示，食用經長期施灌養豬廢水造成銅、鋅累積之稻穀，不致對人體造成危害。

誌謝

本研究廢水與土壤部分分析項目係委託本所農業化學組分析與資訊服務研究室進行分析，特此致謝。

引用文獻

Aldrich, A. P., D. Kistler, and L. Sigg. 2002. Speciation of Cu and Zn in drainage water from agricultural soils. Environ. Sci. Technol. 36:4824–4830. doi:10.1021/es025813x

Cambra, K., T. Martínez, A. Urzelai, and E. Alonso. 1999. Risk analysis of a farm area near a lead- and cadmium-contaminated industrial site. J. Soil Contam. 8:527–540. doi:10.1080/10588339991339450

Chen, C. L., Y. G. Fan, J. L. Zhu, and C. Y. Liao. 2010. Accumulation of copper and zinc from pig manure fertilizer in soil and feed regulation. p.246–255. *in*: Proceedings of the 5th Cross-Strait Conference on Soil and Groundwater Pollution and Remediation. October 25–26, 2010. Taipei, Taiwan. National Taiwan University. Taipei, Taiwan. (in Chinese)

Chen, Z. S., C. C. Tsai, P. T. Wu, C. Y. Lin, C. H. Hsieh, C. M. Huang, ... S. P. Ho. 2004. The effect of applying treated swine wastewater on the soil quality, productivity and quality of sugarcane and pangolagrass. J. Taiwan Livestock Res. 37:105–119. (in Chinese with English abstract)

Health Promotion Administration, Ministry of Health and Welfare (HPA, MOHW). 2018. Sanitation Standard for Contaminants and Toxins in Food. Laws & Regulations Database of the Republic of China. https://law.moj.gov.tw/ENG/LawClass/LawAll.aspx?pcode=L0040138 (visit on 4/23/2019) (in Chinese)

Hill, G. M., G. L. Cromwell, T. D. Crenshaw, C. R. Dove, R. C. Ewan, D. A. Knabe, ... T. L. Veum. 2000. Growth promotion effects and plasma changes from feeding high dietary concentrations of zinc and copper to weanling pigs (regional study). J. Anim. Sci. 78:1010–1016. doi:10.2527/2000.7841010x

Holm, A. 1990. *E. coli* associated diarrhoea in weaner pigs: Zinc oxide added to the feed as a preventative measure. p.154. *in*: Proceedings of the International Pig Veterinary Society, 11th Congress. July 1–5, 1990. Lausanne, Switzerland. Scientific Committee of the 11th I.P.V.S. Congress. Lausanne, Switzerland.

Hu, C. H., K. Xiao, J. Song, and Z. S. Luan. 2013. Effects of zinc oxide supported on zeolite on growth performance, intestinal microflora and permeability, and cytokines expression of weaned pigs. Anim. Feed Sci. Technol. 181:65–71. doi:10.1016/j.anifeedsci.2013.02.003

Järup, L. 2003. Hazards of heavy metal contamination. Br. Med. Bull. 68:167–182. doi:10.1093/bmb/ldg032

Kabata-Pendias, A. and H. Pendias. 1992. Trace Elements in Soils and Plants. 2nd ed. CRC Press. Boca Raton, FL. 365 pp.

Karoline, B. and D. P. Hanne. 2017. The use of zinc and copper in pig production. DCA- Nationalt Center for Jordbrug og Fødevarer. https://pure.au.dk/ws/files/116535883/2017_05_01_Pi (visit on 9/30/2020)

Lucas, S. D. M., S. C. Sampaio, M. A. Uribe-Opazo, S. D. Gomes, N. C. H. Kessler, and N. V. Prado. 2013. Long-term behavior of Cu and Zn in soil and leachate of an intensive no-tillage system under swine wastewater and mineral fertilization. Afr. J. Agric. Res. 8:639–647. doi:10.5897/AJAR12.2063

National Environmental Research Academy (NERA), Ministry of Environment. 2008. Methods for Measuring Soil and Sediment Moisture Content- Gravimetric Method (NIEA S410.62C). https://www.moenv.gov.tw/nera/D650FF755904A079/a2b2399f-c66e-4754-b536-8ee18673f78d (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2013. Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (NIEA M105.01B). https://www.moenv.gov.tw/nera/B75C57336584DBF6/e36699d2-2bff-40ac-b069-eb62fe018ef3 (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2015. Detection Method of Nitrate Nitrogen and Nitrite Nitrogen in Water-Cadimium Reduction Method (NIEA W452.52C). https://www.moenv.gov.tw/nera/32A85B63C9E-C18C0/c592c519-59c1-4958-9cda-e44088182ad3 (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2018. Methods for Heavy Metal Detection in Soil- Microwave Assisted Aqua Regia Digestion (NIEA S301.61B). https://www.moenv.gov.tw/nera/D650FF755904A079/c88ca281-7bdb-4b1a-b8a4-0f4badd590e2 (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2019. Methods for Determining the Hydrogen Ion Concentration Index of Water- Electrometric Measurement (NIEA W424.53A). https://www.moenv.gov.tw/nera/32A85B63C9EC18C0/bccbf164-e8ee-41ce-b628-95a900cee559 (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2020. Detection Method of Ammonia Nitrogen in Water- Phenate Method (NIEA W448.52B). https://www.moenv.gov.tw/nera/32A85B63C9EC18C0/4d3d9246-e2fd-43f4-ae03-4c03b2229bf7 (visit on 5/16/2024) (in Chinese)

National Environmental Research Academy (NERA), Ministry of Environment. 2023. Method for Measuring Conductivity in Water- Conductivity Meter (NIEA W203.52C). https://www.moenv.gov.tw/nera/32A85B63C9EC18C0/4bbb-b61c-2014-4f42-9cf4-cd7990ba5dfa (visit on 5/16/2024) (in Chinese)

Novak, J. M., D. W. Watts, and K. C. Stone. 2004. Copper and zinc accumulation, profile distribution, and crop removal in coastal plain soils receiving long-term, intensive applications of swine manure. Trans. ASAE 47:1513–1522. doi:10.13031/2013.17631

Rhoades, J. D. 1982. Cation exchange capacity. p.149–157. *in*: Methods of Soil Analysis: Part 2 Chemical and Microbiological Properties. 2nd ed. (Page, A. L., R. H. Miller, and D. R. Keeney, eds.) Agronomy Monograph No. 9. American Society of Agronomy, Soil Science Society of America. Madison, WI. 1159 pp. doi:10.2134/agronmonogr9.2.2ed.c8

Rosa, D. M., S. C. Sampaio, P. A. M. Pereira, M. M. Mauli, and R. R. Dos Reis. 2017. Swine wastewater: Impacts on soil, plant, and leachate. Eng. Agríc. 37:928–939. doi:10.1590/1809-4430-eng.agric.v37n5p928-939/2017

Rosen, G. D. and P. A. Roberts. 1996. Comprehensive Survey of the Response of Growing Pigs to Supplementary Copper in Feed. Field Investigations and Nutrition Service. London, UK.

Suzuki, K., M. Waki, T. Yasuda, Y. Fukumoto, K. Kuroda, T. Sakai, ... K. Matsuba. 2010. Distribution of phosphorus, copper and zinc in activated sludge treatment process of swine wastewater. Bioresour. Technol. 101:9399–9404. doi:10.1016/j.biortech.2010.07.014

Taiwan Agricultural Research Institute (TARI). 2013. Method for Determining Soil Organic Matter- Combustion/Infrared Spectroscopy (TARI S201.1B). https://www.tari.gov.tw/sub/form/index-1.asp?Parser=2,27,298,277,,,8570 (visit on 5/16/2024) (in Chinese)

Takada, R. 2005. Reduction of copper and zinc contents in pig feces. Jpn. J. Swine Sci. 42:149–156. (in Japanese) doi:10.5938/youton.42.149

United States Environmental Protection Agency Integrated Risk Information System (US-EPA IRIS). 2006. List of substances on IRIS. https://iris.epa.gov/AtoZ/?list_type=alpha (visit on 9/15/2023)

Wagner, G. J. 1993. Accumulation of cadmium in crop plants and its consequences to human health. Adv. Agron. 51:173–212. doi:10.1016/S0065-2113(08)60593-3

World Health Organization (WHO). 1992. Environmental Health Criteria 134: Cadmium. World Health Organization. Geneva, Switzerland. https://iris.who.int/bitstream/handle/10665/38998/9241571349-eng.pdf?sequence=1andisAllowed=y (visit on 9/30/2020)

Yeh, T. Y. 2002. Renovating technologies of heavy metals contaminated farmland soils. Bimonthly Env. Tr. Pub. 61:3–5. (in Chinese)

Effects of Long-Term Application of Pig Wastewater on Paddy Rice Production and Soil Properties

Hsi-Chia Wu[1], Chia-Jen Liu[2], Ya-Hui Shih[3], Min-Chieh Lin[3], Chung-Yi Liao[4], and Chi-Ling Chen[5,*]

Abstract

Wu, H. C., C. J. Liu, Y. H. Shih, M. C. Lin, C. Y. Liao, and C. L. Chen. 2025. Effects of long-term application of pig wastewater on paddy rice production and soil properties. J. Taiwan Agric. Res. 74(3):303–316.

Livestock wastewater for farmland application has been permitted in Taiwan since 2011. However, concerns remain regarding the potential accumulation of heavy metals in the soil. This study presents findings from a 19-year monitoring period (2004–2023), comparing the effects of applying equivalent amounts of nitrogen, phosphorus, and potassium through chemical fertilizers and swine wastewater. The results showed that long-term irrigation with pig wastewater had little effect on soil pH and electrical conductivity (EC), but it gradually increased the soil organic matter content. Copper from the wastewater accumulated in the soil, with an increase of approximately 6 mg kg^{-1} compared to pre-treatment levels, whereas zinc primarily accumulated in rice straw. Based on a mass balance approach and applying 85% of Taiwan's soil contamination monitoring standards for food crop land as the upper threshold, the estimated safe land use duration can exceed 100 years if the N/Cu and N/Zn ratios in the wastewater are above 180 and 360, respectively. Moreover, reducing the copper and zinc input at the source- namely through feed formulation- can further prolong the period during which livestock wastewater can be safely applied to farmland.

Key word: Copper, Zinc, Farmland reuse, *Oryza sativa*.

Received: January 9, 2025; Accepted: May 26, 2025.
* Corresponding author, e-mail: chiling@tari.gov.gov.tw
1 Research Fellow and Division Director, Agricultural Technology Translation Center, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
2 Assistant Research Fellow, Agricultural Technology Translation Center, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
3 Project Assistants, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
4 Assistant Research Fellow, Crop Environment Section, Taichung District Agricultural Research and Extension Station, Changhua County, Taiwan, ROC.
5 Research Fellow, Agricultural Chemistry Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):317–329 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0009

研究報告

多光譜影像技術用於早期檢測水稻蟲害之研究

曹懷文[1] 黃玉舜[1] 黃守宏[2] 賴柏羽[3] 賴明信[4,*]

摘要

曹懷文、黃玉舜、黃守宏、賴柏羽、賴明信。2025。多光譜影像技術用於早期檢測水稻蟲害之研究。台灣農業研究 74(3):317–329。

本研究針對水稻主要害蟲褐飛蝨，結合人工接種試驗與多光譜影像技術，建立一套可應用於早期預警的蟲害評估模式。試驗中模擬不同褐飛蝨族群密度處理，並使用無人機搭載多光譜相機於田間進行影像監測，蒐集植株光譜資訊。所稱『早期』係指水稻自幼穗分化期後至抽穗期前之期間，約對應二期作水稻蟲害好發時段。分析結果顯示，當常態化差異植生指數 (normalized difference vegetation index; NDVI) 相對於對照組之差值超出自然變異範圍 (± 1*s*) 時，即可視為一種預警訊號，具有辨識褐飛蝨危害初期跡象之潛力，並可作為田間早期監測之初步依據。此外，本研究亦探討田區邊行水稻 (rice near border) 作為對照組替代方案之可行性，以提升實務應用之彈性。研究成果提供傳統農業人工監測以外之替代技術，亦為智慧農業蟲害監測提供可量化之操作依據。

關鍵詞：褐飛蝨、蟲害監測、多光譜、智慧農業。

前言

水稻是全球數十億人口賴以為生的重要糧食作物，其生長過程與產量穩定性直接關係到糧食安全。然而，水稻種植正面臨多重挑戰，除了氣候變遷外，病蟲害更是威脅生產穩定性的主要因素之一。其中，褐飛蝨 (*Nilaparvata lugens* (Stål)) 作為水稻重要害蟲，長期以來對水稻產量造成嚴重損害。其不僅通過吸食汁液導致植株衰弱，亦可傳播植物病毒 (Hibino 1989)，進一步影響產量與米質，對農業生產構成極大挑戰 (Chen & Cheng 1978)。為有效防治褐飛蝨，建立準確且即時的監測方法至關重要。然而傳統蟲害監測多依賴人工觀察與取樣分析，不僅耗時費力，且受人為經驗影響，難以因應大規模區域的高效監測需求。在此背景下，多光譜影像技術近年來已廣泛應用於農業領域，包括作物生長監測 (Bendig *et al*. 2015)、產量預估 (Camenzind & Yu 2024) 及病蟲害檢測 (Kerkech *et al*. 2020) 等。該技術能同時擷取多種光譜波段資訊，有助於掌握作物健康狀況，進而協助識別病蟲害的跡象，為農民提供決策依據，採取防治措施以減少損失。Ghobadifar *et al*. (2016) 利用 SPOT-5 衛星多光譜影像資料證實水稻健康狀態與常態化差異植生指數 (normalized difference vegetation index; NDVI) 之間具有高度統計顯著性 ($P < 0.001$)。Prasannakumar *et al*. (2013) 則結合褐飛蝨損害分級植株與高光譜影像分析，發現波長 665 nm 的紅光反射率與危害等級呈現中度相關 ($r = 0.58$)；而以 NDVI 建構之回歸模型，顯示與危害等級之間具有顯著負相關 ($R^2 = 0.82, P < 0.0001$)。隨後，Prasannakumar *et al*. (2014) 於田間應用研究進一步證明，僅

投稿日期：2025 年 4 月 17 日；接受日期：2025 年 6 月 16 日。
[*] 通訊作者：mhlai@tari.gov.tw
[1] 農業部農業試驗所作物組計畫助理。臺灣 臺中市。
[2] 農業部農業試驗所嘉義農業試驗分所植物保護系副研究員兼系主任。臺灣 嘉義市。
[3] 農業部農業試驗所嘉義農業試驗分所植物保護系助理研究員。臺灣 嘉義市。
[4] 農業部農業試驗所作物組研究員。臺灣 臺中市。

以 NDVI 作為線性回歸模型之預測指標，即可達良好準確性 ($R^2 = 0.76, P < 0.0001$)。惟現有研究多聚焦於太空遙測尺度，著重於分析受害與未受害區域的植生指數或高光譜差異，以用於災損分級與精準施藥。然而此類資料常受限於空間解析度與觀測頻率，難以掌握田間小尺度變異，特別是在地形破碎與田區細碎的臺灣農業環境中，應用效果有限。因此，本研究以臺灣農業環境為例，提出一套基於無人機多光譜影像技術的褐飛蝨早期檢測方法。所稱『早期檢測』，係指水稻自幼穗分化期後至抽穗期前，且尚未出現蝨燒或稻株黃化等明顯可見蟲害特徵之前的階段。此時期亦為二期作水稻褐飛蝨族群易於爆發之關鍵時段，具備進行預警防治的實務價值。藉由多光譜影像處理技術與數據分析方法，建立一套蟲害評估模式，驗證其於早期檢測的可行性，並探索實際應用潛力，期盼此研究能為傳統蟲害監測方法提供準確且高效之替代方案，推動臺灣智慧農業發展，進一步提升病蟲害管理效率與糧食安全保障。

材料與方法

供試褐飛蝨蟲源飼養

本試驗使用之褐飛蝨蟲源飼養於農業部農業試驗所嘉義農業試驗分所溫室中，以水稻品種「台農 67 號」植株作為褐飛蝨之飼料水稻，飼養於鋁製生長箱內 (長 92 cm、寬 47 cm、高 81 cm)，經大量飼養後，取同一日齡之二齡若蟲作為供試蟲源。飼養蟲源所使用之水稻品種「台農 67 號」，該品種屬感蟲型，未具抗性，為國內常用之標準飼料稻品種，可有效維持蟲源族群之穩定性與一致性。

褐飛蝨危害試驗

試驗於 2020 年二期作期間進行 (8–11 月)，試驗田區位置為 24.031850°N, 120.690460°E，田區面積約 0.1 ha。試驗品種為水稻「台南 11 號」，採用機械插秧，行株距為 30 cm × 20 cm。田間管理依據《作物施肥手冊》建議進行，施肥總量為氮 132 kg、磷 72 kg 及鉀 48 kg (以 kg ha^{-1} 計)，基肥施用台肥寶效 39 號有機質複合肥料 ($N–P_2O_5–K_2O$ = 12–18–12，台灣肥料股份有限公司，臺灣臺北市)，追肥與穗肥使用硫酸銨 ($N–P_2O_5–K_2O$ = 21–0–0，台灣肥料股份有限公司，臺灣臺北市)。「台南 11 號」為目前臺灣栽種面積最廣之商業品種，選為實驗標的具代表性且利於後續技術推廣應用。本試驗採用完全隨機設計 (completely randomized design; CRD)，設置 5 個處理組 (A–E) 及 1 個對照組 (CK)，各組設 2 重複，共 12 個實驗小區。小區排列為 3 列 × 4 行之規則格網，各組間依隨機方式配置，以避免空間位置造成之系統性偏差。每一小區內設置 1.8 m × 1.8 m × 1.6 m 之 PVC 管架構成之試驗棚，棚內種植 35 叢水稻，並四周外覆蚊帳以防止褐飛蝨跳離區域，棚架頂端因無人機空拍需求無覆蓋蚊帳。於水稻生育期達到幼穗分化期 (panicle initiation stage) 時，依處理組別接種不同數量之二齡褐飛蝨若蟲 (60、125、250、500 及 1,000 insects hill^{-1})，分別標示為 A 至 E 處理組，CK 組不接種作為對照組。邊行組 (rice near border; BL) 為同一試驗田區中，左右兩側往田區內數第 2 與第 3 行水稻的總合樣本 (n ≒ 1,000)。

稻株多光譜影像收集

本研究使用無人機搭載多光譜相機 MicaSense RedEdge-M (AgEagle Aerial Systems Inc., Wichita, KS, USA) 測量試驗田區植株光譜反射率。RedEdge-M 具備 5 個波段，其中心波長 (center wavelength) 與光譜帶寬 (bandwidth full width at half maximum; bandwidth FWHM) 分別為藍光 (blue: 475 nm/20 nm)、綠光 (green: 560 nm/20 nm)、紅光 (red: 668 nm/10 nm)、紅邊 (red-edge; RE: 717 nm/10 nm) 及近紅外光 (near-infrared; NIR: 840 nm/40 nm)。飛行條件設定為航向與側向重疊率皆 85%，飛行高度距地面 120 m，影像解析度為 8.2 cm pixel^{-1}。飛行前後拍攝反射率校正板 (calibrated reflectance panel, MicaSense, AgEagle Aerial Systems Inc., Wichita, KS, USA)，以利後續影像資料校正。飛行任務執行時間為當地

時間 11:00–13:00，期間為 2020 年 8 月 11 日至 11 月 13 日，共執行 31 次多光譜影像拍攝任務。每次影像皆作為獨立資料點保留，最終共獲得 12 小區 × 31 時間點，共計 372 筆數據，作為建構完整時序變化曲線之依據。

多光譜影像處理

本研究使用 Pix4Dmapper (version 4.5.6) 對多光譜影像進行前處理與拼接，生成正射影像後使用 ArcGIS (version 10.4) 建立 shapefile，並框選需要提取光譜的區域後，將正射影像與 shapefile 載入 R (version 3.6.2) 中進行處理與分析。使用套件涵蓋統計建模 (lme4, doBy)、資料處理 (reshape, plyr, dplyr, data.table)、空間資料分析 (raster, rgdal, sp, gdalutils, shapefiles, maptools, spdep, classInt, velox, lidR)、視覺化呈現 (RColorBrewer)、循環資料統計分析 (CircStats)、影像閾值處理 (autothresholdr)，以及資料集處理 (stats19)。最終將框選區域的光譜值與植生指數匯出為 csv 檔案，用於後續分析。

植生指數之選取與分類

植生指數之選取依據相關文獻綜述 (Bannari *et al*. 1995; Zeng *et al*. 2022) 與資料庫 Index DataBase (https://www.indexdatabase.de/; Henrich *et al*. 2009; Henrich *et al*. 2012) 所提供之資訊，並綜合考量使用普遍性、光譜有效波段限制與公式計算之實用性，篩選出候選植生指數共 27 個 (表 1)。為利後續分析與評估，並考量各指數代表之生理意義，將所選植生指數分類為 4 類：(1) NDVI 系列，該類指數主要由近紅外光與其他波段的比值計算而成，數值落於 ± 1 之間；(2) 環境參數校正，考慮不同環境因子對紅光與近紅外光反射率之影響，藉由引入校正參數提升指數穩定性；(3) 植物體色素相關類，利用植株中特定色素對特定波段之反射特性，推估植體色素含量變化；(4) 未分類，包含具應用潛力但不易歸類之常用指數。

植生指數與褐飛蝨危害相關性建置

數據分析首先以線性回歸評估各植生指

表 1. 本研究所使用之候選植生指數一覽表。

Table 1. List of candidate vegetation indices for this study.

Abbreviation[z]	Name	Formula[y]	Type[x]	Reference
NDVI	Normalized difference vegetation index	(NIR − R)/(NIR + R)	1, Red/NIR	Rouse *et al*. (1974)
NDRE	Normalized difference NIR/red-edge index	(NIR − RE)/(NIR + RE)	1, RE/NIR	Barnes *et al*. (2000)
GNDVI	Normalized difference NIR/green index	(NIR − G)/(NIR + G)	1, Green/NIR	Gitelson *et al*. (1996)
BNDVI	Normalized difference NIR/blue index	(NIR − B)/(NIR + B)	1, Blue/NIR	Yang *et al*. (2004)
NDGR	Normalized difference green-red index	(G − R)/(G + R)	1, VIS	Hunt *et al*. (2005)
NDGB	Normalized difference green-blue index	(G − B)/(G + B)	1, VIS	Hunt *et al*. (2005)
RBNDVI	Red-blue NDVI	(NIR − (R + B))/(NIR + (R + B))	1, VIS/RE	Wang *et al*. (2007)
GBNDVI	Green-blue NDVI	(NIR − (G + B))/(NIR + (G + B))	1, VIS/RE	Wang *et al*. (2007)
EVI	Enhanced vegetation index	2.5(NIR − R)/((NIR + 6R − 7.5B) + 1)	2, VIS/NIR	Huete *et al*. (2002)
EVI2	Enhanced vegetation index 2	2.5(NIR − R)/(NIR + 2.4R + 1)	2, Red/NIR	Jiang *et al*. (2008)
SAVI	Soil adjusted vegetation index	(1 + L) (NIR − R)/(NIR + R + L)[w]	2, Red/NIR	Huete (1988)
OSAVI	Optimized soil adjusted vegetation index	(1 + Y) (NIR − R)/(NIR + R + Y)[v]	2, Red/NIR	Rondeaux *et al*. (1996)

表 1. 本研究所使用之候選植生指數一覽表。(續)

Table 1. List of candidate vegetation indices for this study. (continued)

Abbreviation[z]	Name	Formula[y]	Type[x]	Reference
ARI	Anthocyanin reflectance index	(1/G) − (1/RE)	3, Green/RE	Gitelson *et al*. (2001)
mARI	Modified anthocyanin reflectance index	(G(-1) − RE(-1)) × NIR	3, VIS/NIR	Gitelson *et al*. (2006)
CVI	Chlorophyll vegetation index	(NIR/G) × (R/G)	3, VIS/NIR	Vincini *et al*. (2008)
CCCI	Canopy chlorophyll content index	((NIR − RE)/(NIR + RE))/((NIR − R)/(NIR + R))	3, Red/NIR	El-Shikha *et al*. (2008)
CIgreen	Chlorophyll index green	(NIR/G) (-1)	3, Green/NIR	Gitelson *et al*. (2003)
CIrededge	Chlorophyll index red-edge	(NIR/RE) (-1)	3, RE/NIR	Gitelson *et al*. (2003)
GCC	Green chromatic coordinate	G/(R + G + B)	3, VIS	Cheng *et al*. (2020)
LCI	Leaf chlorophyll index	(NIR − RE)/(NIR + R)	3, VIS/NIR	Datt (1999)
MCARI	Modified chlorophyll absorption in reflectance index	((RE − R) − 0.2(RE − G)) × (RE/R)	3, VIS/RE	Daughtry *et al*. (2000)
MCARI1	Modified chlorophyll absorption in reflectance index 1	1.2 × (2.5(NIR − R) − 1.3(NIR − G))	3, VIS/RE	Haboudane *et al*. (2004)
TCARI	Transformed chlorophyll absorption in reflectance index	3((RE − R) − 0.2(RE − G) × (RE/R))	3, VIS/RE	Haboudane *et al*. (2002)
SR	Simple ratio	(NIR/R)	4, Red/NIR	Tucker (1979)
mSR	Modified simple ratio	(NIR/R − 1)/sqrt(NIR/R + 1)	4, VIS/NIR	Chen (1996)
GLI	Green leaf index	(2G − R − B)/(2G + R + B)	4, VIS	Louhaichi *et al*. (2001)
VARIgreen	Visible atmospherically resistant index green	(G − R)/(G + R – B)	4, VIS	Gitelson *et al*. (2002)

[z] NDVI: normalized difference vegetation index; NDRE: normalized difference red edge index; GNDVI: green normalized difference vegetation index; BNDVI: blue normalized difference vegetation index; NDGR: normalized difference green-red index; NDGB: normalized difference green-blue index; RBNDVI: red-blue normalized difference vegetation index; GBNDVI: green-blue normalized difference vegetation index; EVI: enhanced vegetation index; EVI2: two-band enhanced vegetation index; SAVI: soil adjusted vegetation index; OSAVI: optimized soil adjusted vegetation index; ARI: anthocyanin reflectance index; mARI: modified anthocyanin reflectance index; CVI: chlorophyll vegetation index; CCCI: canopy chlorophyll content index; CIgreen: chlorophyll index green; CIrededge: chlorophyll index red edge; GCC: green chromatic coordinate; LCI: leaf chlorophyll index; MCARI: modified chlorophyll absorption ratio index; MCARI1: modified chlorophyll absorption ratio index 1; TCARI: transformed chlorophyll absorption ratio index; SR: simple ratio; mSR: modified simple ratio; GLI: green leaf index; VARIgreen: visible atmospherically resistant index green.

[y] The spectral bands referenced in the formula column are blue (B), green (G), red (R), red-edge (RE), and near-infrared (NIR).

[x] VIS is an abbreviation for visible spectroscopy. The numbers in the type column represent classification types in this study: 1. NDVI series, 2. environmental reflectance adjustment, 3. pigment content, and 4. unclassified.

[w] The correction factor used in the formula is Y = 0.16.

[v] The correction factor used in the formula is L = 0.75.

數 (表 1) 與水稻生長狀況之相關性，排除呈非線性相關之指數；隨後以決定係數 (coefficient of determination, R^2) 大於 0.9 作為篩選門檻，選取主要候選指數。為評估處理組與對照組間 NDVI 差異是否超出自然變異範圍，本研究以對照組內部變異為基礎，設定 ± 0.0135 為自然變異門檻，據以建立 NDVI 差值熱圖，呈現各處理組於各時間點相對於對照組的偏離狀況。進一步為補強差異穩定性之判斷，採用 bootstrap 重抽樣法對處理組與對照組之 NDVI 差值進行 1,000 次有放回抽樣，估算平均差異超出自然變異門檻之機率，作為處理效應穩定性之補充指標。另針對所選指數，亦進行配對樣本 *t* 檢定 (paired samples *t*-test) 以分析處理組與對照組間之統計差異，惟該檢定僅作為輔助性分析，並非處理效果判定之依據。所有統計計算與視覺化均於 R (version 3.6.2) 環境中進行，並使用基本統計與圖形套件協助資料處理與呈現。

結果與討論

水稻「台南 11 號」生育期之 NDVI 自然變異特性

為進行異常狀況之判別，須先確定水稻在慣行耕作條件下的生長自然變異範圍，以作為後續分析與比較之依據。紅光與近紅外光波段 (red-NIR) 所構成的植生指數，為目前應用最廣泛的指標類型之一。故本研究首先針對「台南 11 號」水稻於全生育期內的 NDVI 變化進行分析，並繪製其時序趨勢圖，以描繪各生育階段之自然變異特徵 (圖 1)。結果顯示，NDVI 從插秧後至營養生長期快速上升，於幼穗分化期前後達到峰值；孕穗期間則趨於平穩，抽穗期開始後則逐步下降。顯示水稻於孕穗期間，植株生長勢呈現緩幅波動。本研究後續針對異常狀況之分析，將以此期間的自然變異範圍作為判別基準，該時段亦與前言所述之『早期檢測』目標相符，具備實務應用意義。

NDVI 應用於水稻健康狀態識別與蟲害發生偵測之可行性

在未受外力干擾的情況下，水稻田區間之生長勢理應具備一致性。為建立異常值偵測依據，本研究透過量化 NDVI 的自然變異程度作為判定基準。研究中選取對照組水稻在插秧後 50–80 d 的 NDVI 資料，計算每日標準差的平均值 (± 1*s*; *s* = 0.0135)，做為自然變異閾值。當實驗組 NDVI 與對照組之差異超過此閾值，即視為可能受到蟲害影響。如圖 2 所示，接種褐飛蝨後，各處理組的 NDVI 差異 (ΔNDVI) 呈現不同程度的下降趨勢，且與蟲口密度呈

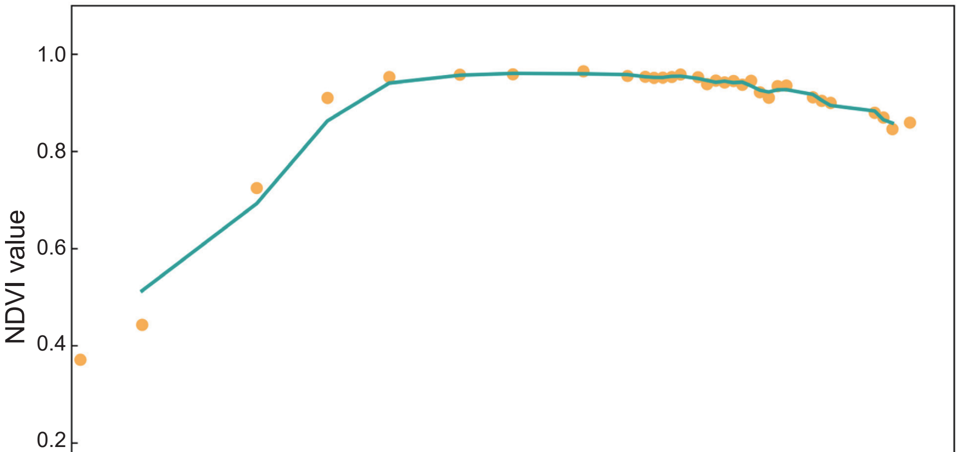

圖 1. 「台南 11 號」水稻全生育時期之常態化差異植生指數 (normalized difference vegetation index; NDVI) 變化。X 軸為插秧後天數，Y 軸為 NDVI 數值。圖中呈現 2 類資料：實際觀測之 NDVI 值 (以點表示) 與經簡單移動平均 (simple moving average; SMA) 處理後的趨勢線 (以線段表示)。結果顯示，NDVI 由秧苗期至幼穗分化期逐漸上升，於幼穗分化期至孕穗期趨於穩定，並在孕穗後期略為下降。反映水稻不同生育階段之反射光譜特性變化。

Fig. 1. Temporal changes in normalized difference vegetation index (NDVI) for the rice cultivar ‘Tainan 11’ throughout the entire growth period. The x-axis represents days after transplanting, and the y-axis indicates NDVI values. Two types of data are presented: observed NDVI values (dots) and trend lines derived from simple moving average (SMA) smoothing (lines). The results show that NDVI increased gradually from the seedling stage to the panicle initiation stage, remained stable through the booting stage, and slightly declined during the late booting stage. This pattern indicates the spectral reflectance characteristics associated with different growth stages of rice.

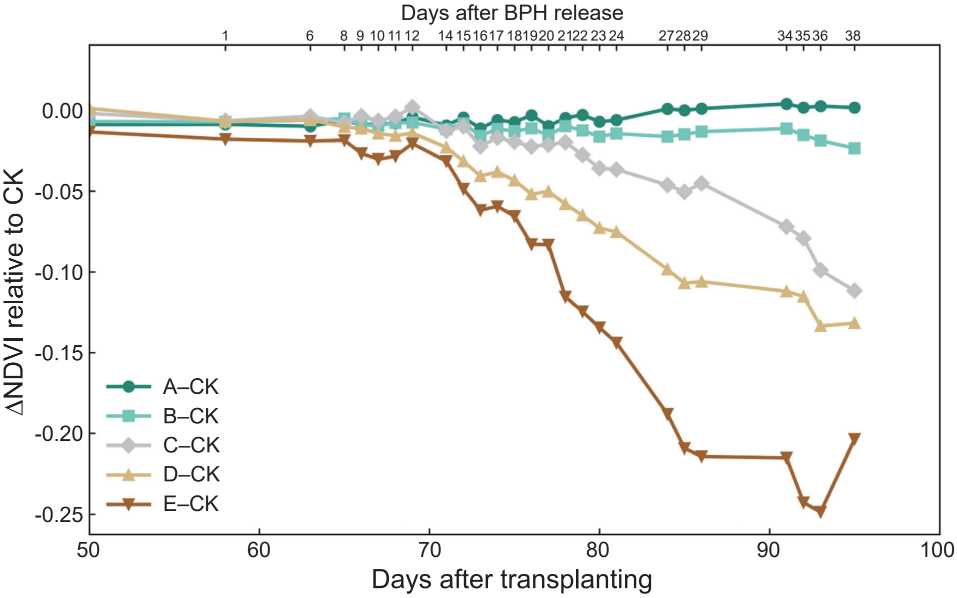

圖 2. 不同褐飛蝨密度危害下「台南 11 號」水稻生長勢之變化。本圖顯示不同接蟲密度處理下，水稻常態化差異植生指數 (normalized difference vegetation index; NDVI) 差異值隨時間之變化趨勢。X 軸下方為插秧後天數，上方為褐飛蝨釋放後天數；Y 軸為處理組相對於對照組之 NDVI 差異值。實驗組包含對照組 (CK) 與 5 種接蟲密度處理組，分別為 A (60 insect hill^{-1})、B (125 insect hill^{-1})、C (250 insect hill^{-1})、D (500 insect hill^{-1}) 及 E (1,000 insect hill^{-1})。

Fig. 2. Growth response of ‘Tainan 11’ rice under different brown planthopper (BPH) infestation densities. The figure illustrates temporal trends in normalized difference vegetation index (NDVI) differences between treatment and control groups under varying BPH densities. The lower x-axis indicates days after transplanting, while the upper x-axis represents days after BPH release. The y-axis shows the NDVI deviation relative to the control. Experimental treatments include a control group (CK) and five infestation densities: A (60 insects hill^{-1}), B (125 insects hill^{-1}), C (250 insects hill^{-1}), D (500 insects hill^{-1}), and E (1,000 insects hill^{-1}).

正相關。處理組 E–CK (1,000 insect hill^{-1}) 在接蟲後 1–3 d 即快速下降，最終 ΔNDVI 降幅超過 0.25，顯示其受害程度最為嚴重；D–CK (500 insect hill^{-1}) 與 C–CK (250 insect hill^{-1}) 則分別於接蟲後約 10 d 與 15 d 開始明顯偏離對照組。相較之下，A–CK 與 B–CK 所呈現的差異幅度有限，ΔNDVI 變化接近零。此圖清楚反映褐飛蝨蟲口密度對水稻生長勢的累積性影響。進一步以熱圖觀察 (圖 3A)，CK–E 組在接蟲後 1 d (插秧後 58 d)、CK–D 在接蟲後 10 d (插秧後 67 d)、CK–C 組在接蟲後 16 d (插秧後 73 d) 即已超過變化閾值，顯示生長勢異常開始發生。為評估這些差異是否具有統計穩定性，本研究進一步針對各處理組進行 bootstrap 重抽樣分析 (n = 1,000)。結果顯示，隨處理強度由 A 組至 E 組遞增，超出門檻的平均機率亦由 27.4% 增至 81.5% (表 2)，呈現與熱圖相符之趨勢。此結果強化了熱圖 (圖 3A) 所示的觀察結論，證明視覺上偏離門檻較大的區域 (棕色)，在統計上亦具有較高的出現機率，具實質解釋意義。為系統性評估各處理組 (A–E) 與對照組 (CK) 間 NDVI 表現之差異，本研究針對對照組 (CK-I 與 CK-II) 分別與各處理組子組 (例如 A-I 與 A-II) 進行 4 組配對 t 檢定 (four pairwise t-tests)，並彙整其最小 t 值、最大 P 值、平均效果量 (Cohen’s d) 及 Fisher 合併 P 值，以評估處理效應之顯著性與一致性。結果顯示 (表 2)，處理組 C 與 E 及 CK 間之差異最為穩定且顯著，4 組配對檢定皆達顯著標準 (最大 P 值分別為 0.006 與

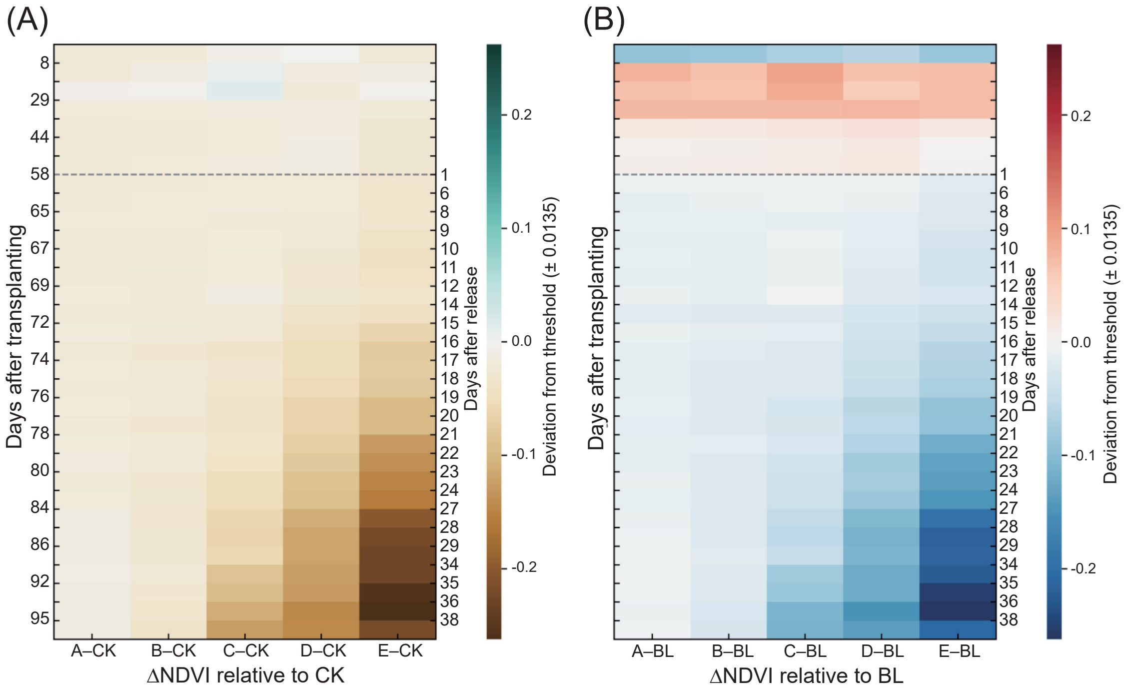

圖 3. 各接蟲密度處理組相對於對照組與邊行組之常態化差異植生指數 (normalized difference vegetation index; NDVI) 偏離程度熱圖。(A) 顯示處理組相對於對照組 (CK) 之 NDVI 差異值；(B) 顯示處理組相對於邊行組 (BL) 之差異值。X 軸表示各處理組別；Y 軸左側為插秧後天數，右側為釋放褐飛蝨後天數，灰色虛線表示接蟲日。色階表示 ΔNDVI 偏離自然變異閾值 (± 0.0135) 之程度，採對稱色階配置。此圖呈現不同接蟲密度處理下水稻生長勢變化之時間動態與影響強度，視覺化呈現以自然變異閾值判斷水稻受害程度與預警時間之決策參考。

Fig. 3. Heatmaps of normalized difference vegetation index (NDVI) deviation under different brown planthopper (BPH) infestation densities relative to the control and rice near border (BL) groups. (A) shows the ΔNDVI of each treatment group relative to the control (CK), and (B) shows the ΔNDVI relative to the rice near border group (BL). The x-axis represents treatment groups; the left y-axis indicates days after transplanting, and the right y-axis indicates days after BPH release. The gray dashed line marks the infestation day. The color scale represents the degree of deviation from the natural variation threshold (± 0.0135), with a symmetric diverging palette. These heatmaps visualize the temporal dynamics and severity of rice growth suppression under different infestation densities, providing a reference for damage assessment and early-warning decisions based on the natural variation threshold.

0.0001)，平均 Cohen's d 效果量亦分別達 0.68 與 0.98，屬中至大型效果。相較之下，A 組與 B 組雖經 Fisher 合併 P 值亦顯著 ($P < 10^{-10}$)，惟其個別配對檢定結果不一致 (最大 P 值分別高達 0.376 與 0.728)，且平均效果量相對偏低，顯示處理效應穩定性不足。綜上所述，本研究透過 NDVI 的量化分析結合自然變異閾值判別法，建立了水稻生長異常之合理判別依據，並進一步驗證其在蟲害影響下的實用性與穩定性，展現此方法於田間監測與植株健康評估上的應用潛力。

稻田邊行稻株的光譜值作為對照組參照之可行性評估

在產業利用上，要找到可以作為『對照組』的稻株並不容易，若設立『對照田』又擔心管理或土壤質地差異，造成干擾。使用田區的邊行稻株光譜作為對照組，可以將干擾因子減至最低，可能是解決此問題的有效方法。此方法乃基於褐飛蝨習性的觀察，當褐飛蝨在棲息田區時，通常會選擇稻田內部不透風的地方聚集，通風的邊行稻株受到蟲害較少。具體而

表 **2.** 對照組 (CK) 與處理組 (A–E) 之間的常態化差異植生指數 (normalized difference vegetation index; NDVI) 差異統計摘要。

Table 2. Summary statistics of normalized difference vegetation index (NDVI) differences between control and treatment groups.

Group[z]	Boot[y]	Min *t* [x]	Max *P* [w]	Mean d[v]	Fisher *P* [u]	Significance[t]
A	27.4%	0.90	0.3760	0.40	7.85×10^{-19}	***
B	32.1%	0.35	0.7280	0.91	3.40×10^{-17}	***
C	54.5%	2.93	0.0063	0.68	3.02×10^{-10}	**
D	63.4%	0.05	0.9580	0.76	2.25×10^{-13}	***
E	81.5%	4.49	0.0001	0.98	6.07×10^{-17}	***

[z] Each treatment group (e.g., A-I and A-II) was evaluated through four pairwise comparisons against the control groups (CK-I and CK-II).
[y] Proportion of bootstrap replicates from 1,000 resampling comparisons between each treatment group (A–E) and the control (CK) at matched time points (50–80 d after transplanting; DAT). A higher proportion indicates more consistent NDVI deviation induced by treatment effects.
[x] The smallest absolute *t*-value among the four CK–treatment pairwise comparisons, representing the weakest statistical support among the four tests.
[w] The largest *P*-value among the four CK–treatment pairwise comparisons. A higher value may indicate inconsistent treatment effects across replicates.
[v] The mean Cohen's d across the four CK–treatment pairwise comparisons was used to estimate the average effect size. According to Cohen's guidelines, values above 0.8 represent large effects, while values around 0.5 indicate moderate differences.
[u] To evaluate the overall treatment effect across the four CK–treatment pairwise comparisons, Fisher's combined probability test was applied. This method yields a single *P*-value reflecting the joint significance of all comparisons.
[t] Significance codes: $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$, based on Fisher's combined *P*-value across the four pairwise tests.

言，本研究將田區邊行的第 2、3 行稻株光譜作為對照，通過測量這些邊行稻株的光譜值，計算其植生指數的平均值，並將其與田區內部的對照組進行比較，評估邊行稻株作為對照組的可行性 (圖 4)。結果顯示，邊行組 (BL) 在生育初期的生長勢較對照組 (CK) 平均值低，顯示其生長早期受邊際效應影響。然而，在本研究設定的自然變異閾值時期 (插秧後 50–80 d)，邊行組之 NDVI 數值皆落於區間內，顯示其在此階段的生長趨勢與對照組趨於一致。因此，即使邊際效應導致生長早期具落差，後期仍能穩定落入標準閾值範圍 (± 1*s*; *s* = 0.0135)，不影響作為基準時的判別準確性 (圖 3B)。通過這種方法，能夠在無需明確對照組的情況下，利用邊行作為對照，有效判斷水稻是否受到褐飛蝨的危害。此方式不僅提高田間蟲害檢測的靈活性與可靠性，也為精準農業提供新的技術手段。

討論

本研究透過評估水稻生育期間植生指數的時序變化模式，初步探討其在蟲害檢測上的應用潛力，並提出一種基於 NDVI 自然變異閾值的簡易判別方式，用於區分健康與受蟲害影響之稻株。多項植生指數 (如 canopy chlorophyll content index (CCCI)、leaf chlorophyll index (LCI)、NDVI、normalized difference red edge index (NDRE) 及 modified simple ratio (mSR)) 與水稻生長勢呈現良好相關性 (表 3)，以 NDVI 為例，其在孕穗期間變化穩定 (圖 1)，並於接蟲處理後，部分實驗組能明顯觀察到呈現下降趨勢 (圖 2)，顯示其對異常生長具備敏感性。基於上述觀察，本研究提出一項基於自然變異的 NDVI 閾值判別法，簡化多光譜影像於水稻生長異常鑑別上的應用流程。於本研究案例中，此閾值能區分出 NDVI 偏低之區域 (圖 3)，顯示其在蟲害早期預警上具潛在應用價值，惟仍需進一步驗證其穩定性與通用性。前人研究中，Prasannakumar *et al*. (2013) 發現波長 665 nm 的紅光反射率可反映褐飛蝨危害，Ghobadifar *et al*. (2016) 亦指出 NDVI 能識別水稻健康狀態。本研究選擇延續前述紅光波段植生指數之應用架構，雖監測尺度由衛星遙測轉為無人機近地觀測，但所得結果仍與上述研究發現一致。惟本研究聚焦於受害初期之檢測潛力，因此僅以不同密度的蟲口數模擬初期可

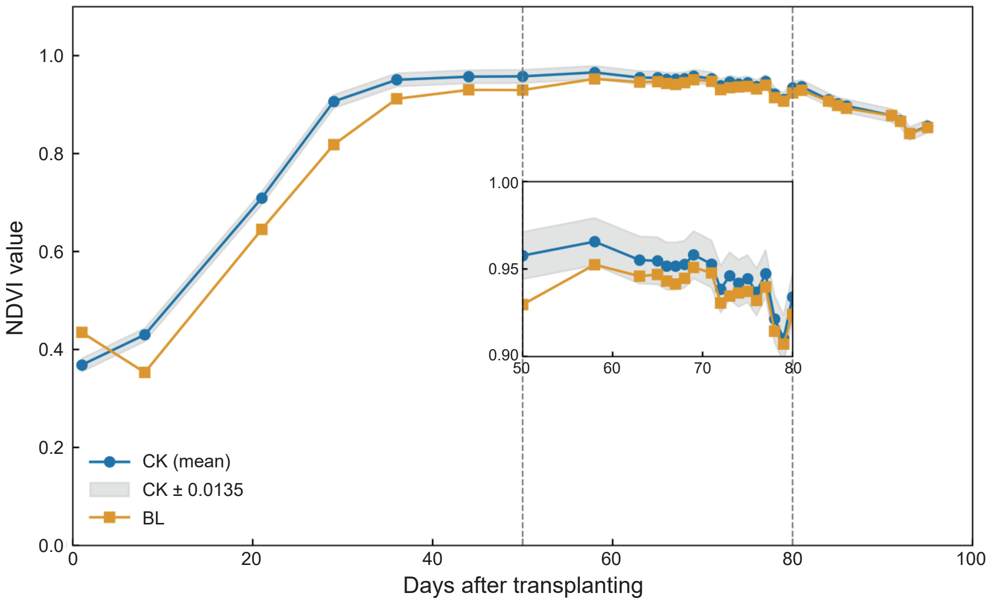

圖 4. 「台南 11 號」對照組與邊行組於全生育期間之常態化差異植生指數 (normalized difference vegetation index; NDVI) 變化趨勢。X 軸為插秧後天數，Y 軸為 NDVI 數值。藍線表示對照組 (CK) 之平均 NDVI，灰色區域為自然變異閾值範圍 (± 0.0135)；橘線為邊行組 (BL) 之 NDVI。虛線為計算自然變異閾值所採用之時間範圍 (50–80 d after transplanting; DAT)。圖中另嵌入局部放大圖，以突顯該期間兩組之 NDVI 細部變化。

Fig. 4. Temporal trends of normalized difference vegetation index (NDVI) for the control group and rice near border throughout the growth period of 'Tainan 11'. The x-axis represents days after transplanting, and the y-axis indicates NDVI values. The blue line shows the mean NDVI of the control group (CK), and the shaded gray area represents the natural variation threshold (± 0.0135); the orange line represents the NDVI of rice near border. Dashed lines indicate the time range (50–80 d after transplanting; DAT) used to calculate the natural variation threshold. An inset panel highlights the detailed NDVI differences between the two groups during this period.

能之受害狀況，並未對試驗組水稻進行危害分級。此設計係基於早期預警之應用場景出發考量，若受害狀況已明顯至可被分級，則多已失去提早介入防治之意義。統計檢定結果雖顯示各處理組間具顯著差異 (表 2)，惟受限於本研究每處理組僅設置 2 重複樣區 (n = 2)，其統計推論力相當有限。本文所呈現之配對 t 檢定結果，僅供觀察趨勢與差異方向之參考，並未據以作為支持本研究結論之依據。為補強統計推論力，本文進一步採用 bootstrap 重抽樣技術，模擬各處理組間 NDVI 分布之穩定趨勢與處理強度差異。重抽樣以對照組 NDVI 值的自然變異 (即樣本標準差 s = 0.0135) 為基礎，生成模擬分布以反映不同處理組可能出現的變異幅度。結果顯示，各處理組於重複抽樣下呈現一致且具層次之機率分布趨勢 (表 2)，顯示其與實際蟲口密度設定具一致性。雖無傳統統計檢定之顯著性支持，該結果仍提供本方法於辨識處理強度差異上的潛在可行性，亦有助於彌補小樣本設計所造成之推論限制。為提升本方法之實務應用潛力，本研究提出以本田邊行水稻當作對照組替代基準的可行性 (圖 3 與圖 4)。分析結果顯示，在本實驗設定的防治預警時期 (50–80 d after transplanting; DAT) 內，邊行組之 NDVI 數值大致落於對照組 NDVI 的 1 個樣本標準差 (± 1s) 範圍內，初步指出在目前資料量有限的情況下，對邊行組 (BL) 與對照組 (CK) 於數值表現上具有一定程度相似性，顯示其作為替代比較基準的潛力。綜上所述，本研究所提出之基於 NDVI 自然變異的判別方法，於中高蟲口密度 (> 250 insect hill^{-1}) 處理條件下展現初步可行性；此外，將本田邊行組作為田間參照標準的可能性，亦為後續實務操作提供可行的應用途徑。然而，目前整體方法

表 3.　「台南 11 號」全生育時期的植生指數二次多項式回歸模型之決定係數。

Table 3.　The coefficient of determination (R^2) of the quadratic polynomial regression model for vegetation indices across the full growth period of ‘Tainan 11’.

Abbreviation[z]	Coefficient of determination (R^2)	
	Replicate 1	Replicate 2
NDVI	0.93	0.94
NDRE	0.93	0.92
GNDVI	0.62	0.59
BNDVI	0.75	0.71
NDGR	0.49	0.41
NDGB	0.48	0.48
RBNDVI	0.88	0.88
GBNDVI	0.69	0.67
EVI	0.41	0.37
EVI2	0.42	0.39
SAVI	0.39	0.42
OSAVI	0.47	0.51
ARI	0.21	0.25
mARI	0.68	0.67
CVI	0.53	0.41
CCCI	0.91	0.93
CIgreen	0.76	0.77
CIrededge	0.81	0.78
GCC	0.63	0.55
LCI	0.95	0.94
MCARI	0.41	0.37
MACRI1	0.41	0.36
TCARI	0.59	0.73
SR	0.83	0.81
mSR	0.93	0.91
GLI	0.66	0.59
VARIgreen	0.14	0.16

[z] NDVI: normalized difference vegetation index; NDRE: normalized difference red edge index; GNDVI: green normalized difference vegetation index; BNDVI: blue normalized difference vegetation index; NDGR: normalized difference green-red index; NDGB: normalized difference green-blue index; RBNDVI: red-blue normalized difference vegetation index; GBNDVI: green-blue normalized difference vegetation index; EVI: enhanced vegetation index; EVI2: two-band enhanced vegetation index; SAVI: soil adjusted vegetation index; OSAVI: optimized soil adjusted vegetation index; ARI: anthocyanin reflectance index; mARI: modified anthocyanin reflectance index; CVI: chlorophyll vegetation index; CCCI: canopy chlorophyll content index; CIgreen: chlorophyll index green; CIrededge: chlorophyll index red edge; GCC: green chromatic coordinate; LCI: leaf chlorophyll index; MCARI: modified chlorophyll absorption ratio index; MCARI1: modified chlorophyll absorption ratio index 1; TCARI: transformed chlorophyll absorption ratio index; SR: simple ratio; mSR: modified simple ratio; GLI: green leaf index; VARIgreen: visible atmospherically resistant index green.

對於低蟲口密度 (< 125 insect $hill^{-1}$) 受害情形的辨識能力仍待強化，後續仍需進一步驗證其穩定性與通用性，以支撐其在實際監測情境中的廣泛應用。若本研究所揭示之初步成果與方法方向獲得認可，則以下 3 個發展策略應值得進一步推進與支持：首先，擬擴充試驗資料至多個生長期與年度，藉以建立具時序穩定性的 NDVI 自然變異閾值範圍，強化模型在不同生

育條件下之泛用性。其次，考慮導入空間統計方法 (如空間自相關指標與局部離群值分析等) 與自適應門檻演算法，以強化植株異常區之空間判別能力，進一步提升預警模型對田間異常分布的感知敏銳度。第三，未來將探索以空間異常偵測取代時序分析之可能性，特別是在高頻率觀測條件受限之情境中，建立以同時期樣區為基礎之異常判別架構。整體而言，本策略於近程可提供具實用性的多光譜蟲害預警參數，中程可藉使用者回報資料擴充數據庫、提升適用範圍，並於長程以空間分析取代時序分析，有效提升處理效率並降低技術成本。

引用文獻

Bannari, A., D. Morin, F. Bonn, and A. R. Huete. 1995. A review of vegetation indices. Remote Sens. Rev. 13:95–120. doi:10.1080/02757259509532298

Barnes, E. M., T. R. Clarke, S. E. Richards, P. D. Colaizzi, J. Haberland, M. Kostrzewski, ... M. S. Moran, 2000. Coincident detection of crop water stress, nitrogen status and canopy density using ground-based multispectral data. *in*: Proceedings of the Fifth International Conference on Precision Agriculture. July 16–19, 2000. Bloomington, MN. American Society of Agronomy, Crop Science Society of America, and Soil Science Society of America. Madison, WI.

Bendig, J., K. Yu, H. Aasen, A. Bolten, S. Bennertz, J. Broscheit, ... G. Bareth. 2015. Combining UAV-based plant height from crop surface models, visible, and near infrared vegetation indices for biomass monitoring in barley. Int. J. Appl. Earth Obs. Geoinf. 39:79–87. doi:10.1016/j.jag.2015.02.012

Camenzind, M. P. and K. Yu. 2024. Multi temporal multispectral UAV remote sensing allows for yield assessment across European wheat varieties already before flowering. Front. Plant Sci. 14:1214931. doi:10.3389/fpls.2023.1214931

Chen, C. N. and C. C. Cheng. 1978. The population levels of *Nilaparvata lugens* (Stål) in relation to the yield loss of rice. I. Plant Prot. Bull. Taiwan 20:197–209.

Chen, J. M. 1996. Evaluation of vegetation indices and a modified simple ratio for boreal applications. Can. J. Remote Sens. 22:229–242. doi:10.1080/07038992.1996.10855178

Cheng, R., T. S. Magney, D. Dutta, D. R. Bowling, B. A. Logan, S. P. Burns, ... C. Frankenberg. 2020. Decomposing reflectance spectra to track gross primary production in a subalpine evergreen forest. Biogeosciences 17:4523–4544. doi:10.5194/bg-17-4523-2020

Datt, B. 1999. A new reflectance index for remote sensing of chlorophyll content in higher plants: tests using *Eucalyptus* leaves. J. Plant Physiol. 154:30–36. doi:10.1016/S0176-1617(99)80314-9

Daughtry, C. S. T., C. L. Walthall, M. S. Kim, E. B. de Colstoun, and J. E. McMurtrey III. 2000. Estimating corn leaf chlorophyll concentration from leaf and canopy reflectance. Remote Sens. Environ. 74:229–239. doi:10.1016/S0034-4257(00)00113-9

El-Shikha, D. M., E. M. Barnes, T. R. Clarke, D. J. Hunsaker, J. A. Haberland, P. J. Pinter Jr., ... T. L. Thompson. 2008. Remote sensing of cotton nitrogen status using the canopy chlorophyll content index (CCCI). Trans. Am. Soc. Agric. Eng. 51:73–82. doi:10.13031/2013.24228

Ghobadifar, F., W. Aimrun, and M. N. Jebur. 2016. Development of an early warning system for brown planthopper (BPH) (*Nilaparvata lugens*) in rice farming using multispectral remote sensing. Precis. Agric. 17:377–391. doi:10.1007/s11119-015-9422-9

Gitelson, A. A., Y. J. Kaufman, and M. N. Merzlyak. 1996. Use of a green channel in remote sensing of global vegetation from EOS-MODIS. Remote Sens. Environ. 58:289–298. doi:10.1016/S0034-4257(96)00072-7

Gitelson, A. A., Y. J. Kaufman, R. Stark, and D. Rundquist. 2002. Novel algorithms for remote estimation of vegetation fraction. Remote Sens. Environ. 80:76–87. doi:10.1016/S0034-4257(01)00289-9

Gitelson, A. A., G. P. Keydan, and M. N. Merzlyak. 2006. Three-band model for noninvasive estimation of chlorophyll, carotenoids, and anthocyanin contents in higher plant leaves. Geophys. Res. Lett. 33:L11402. doi:10.1029/2006GL026457

Gitelson, A. A., M. N. Merzlyak, and O. B. Chivkunova. 2001. Optical properties and nondestructive estimation of anthocyanin content in plant leaves. Photochem. Photobiol. 74:38–45. doi:10.1562/0031-8655(2001)0740038OPANEO2.0.CO2

Gitelson, A. A., A. Viña, T. J. Arkebauer, D. C. Rundquist, G. Keydan, and B. Leavitt. 2003. Remote estimation of leaf area index and green leaf biomass in maize canopies. Geophys. Res. Lett. 30:1248. doi:10.1029/2002GL016450

Haboudane, D., J. R. Miller, E. Pattey, P. J. Zarco-Tejada, and I. B. Strachan. 2004. Hyperspectral vegetation indices and novel algorithms for predicting green LAI of crop canopies: Modeling and validation in the context of precision agriculture. Remote Sens. Environ. 90:337–352. doi:10.1016/j.rse.2003.12.013

Haboudane, D., J. R. Miller, N. Tremblay, P. J. Zarco-Tejada, and L. Dextraze. 2002. Integrated narrow-band vegetation indices for prediction of crop chlorophyll content for application to precision agriculture. Remote Sens. Environ. 81:416–426. doi:10.1016/S0034-4257(02)00018-4

Henrich, V., C. Götze, A. Jung, C. Sandow, D. Thürkow, and C. Gläßer. 2009. Development of an online indices database: Motivation, concept and implementation. p.16–18. *in*: Proceedings of the 6th EARSeL Imaging Spectroscopy SIG Workshop Innovative Tool for Scientific and Commercial Environment Applications. March 16–19, 2009. Tel Aviv, Israel. European Association of Remote Sensing Laboratories. Strasbourg, France.

Henrich, V., G. Krauss, C. Götze, and C. Sandow. 2012. IDB-www.indexdatabase.de Entwicklung einer Datenbank für Fernerkundungsindizes: Ziele und Eigenschaften der IDB. AK Fernerkundung. Tech. Rep. Bochum, Germany. (in German)

Hibino, H. 1989. Insect-borne viruses of rice. p.209–241. *in*: Advances in Disease Vector Research. (K. F. Harris, ed.) Springer. New York. 412 pp. doi:10.1007/978-1-4612-3292-6_8

Huete, A. R. 1988. A soil-adjusted vegetation index (SAVI). Remote Sens. Environ. 25:295–309. doi:10.1016/0034-4257(88)90106-X

Huete, A., K. Didan, T. Miura, E. P. Rodriguez, X. Gao, and L. G. Ferreira. 2002. Overview of the radiometric and biophysical performance of the MODIS vegetation indices. Remote Sens. Environ. 83:195–213. doi:10.1016/S0034-4257(02)00096-2

Hunt, E. R., Jr., M. Cavigelli, C. S. T. Daughtry, J. E. McMurtrey III, and C. L. Walthall. 2005. Evaluation of digital photography from model aircraft for remote sensing of crop biomass and nitrogen status. Precis. Agric. 6:359–378. doi:10.1007/s11119-005-2324-5

Jiang, Z., A. R. Huete, K. Didan, and T. Miura. 2008. Development of a two-band enhanced vegetation index without a blue band. Remote Sens. Environ. 112:3833–3845. doi:10.1016/j.rse.2008.06.006

Kerkech, M., A. Hafiane, and R. Canals. 2020. Vine disease detection in UAV multispectral images using optimized image registration and deep learning segmentation approach. Comput. Electron. Agric. 174:105446. doi:10.1016/j.compag.2020.105446

Louhaichi, M., M. M. Borman, and D. E. Johnson. 2001. Spatially located platform and aerial photography for documentation of grazing impacts on wheat. Geocarto Intl. 16(1):65–70. doi:10.1080/10106040108542184

Prasannakumar, N. R., S. Chander, and R. N. Sahoo. 2014. Characterization of brown planthopper damage on rice crops through hyperspectral remote sensing under field conditions. Phytoparasitica 42:387–395. doi:10.1007/s12600-013-0375-0

Prasannakumar, N. R., S. Chander, R. N. Sahoo, and V. K. Gupta. 2013. Assessment of brown planthopper, (*Nilaparvata lugens*) [Stål], damage in rice using hyperspectral remote sensing. Int. J. Pest Manag. 59:180–188. doi:10.1080/09670874.2013.808780

Rondeaux, G., M. Steven, and F. Baret. 1996. Optimization of soil-adjusted vegetation indices. Remote Sens. Environ. 55:95–107. doi:10.1016/0034-4257(95)00186-7

Rouse, J. W., Jr., R. H. Haas, D. W. Deering, J. A. Schell, and J. C. Harlan. 1974. Monitoring the vernal advancement and retrogradation (green wave effect) of natural vegetation. NASA. Tech. Rep. E75-10354. Washington, DC. 390 pp.

Tucker, C. J. 1979. Red and photographic infrared linear combinations for monitoring vegetation. Remote Sens. Environ. 8:127–150. doi:10.1016/0034-4257(79)90013-0

Vincini, M., E. Frazzi, and P. D’Alessio. 2008. A broadband leaf chlorophyll vegetation index at the canopy scale. Precis. Agric. 9:303–319. doi:10.1007/s11119-008-9075-z

Wang, F. M., J. F. Huang, Y. L. Tang, and X. Z. Wang. 2007. New vegetation index and its application in estimating leaf area index of rice. Rice Sci. 14:195–203. doi:10.1016/S1672-6308(07)60027-4

Yang, C., J. H. Everitt, J. M. Bradford, and D. Murden. 2004. Airborne hyperspectral imagery and yield monitor data for mapping cotton yield variability. Precis. Agric. 5:445–461. doi:10.1007/s11119-004-5319-8

Zeng, Y., D. Hao, A. Huete, B. Dechant, J. Berry, J. M. Chen, ... M. Chen. 2022. Optical vegetation indices for monitoring terrestrial ecosystems globally. Nat. Rev. Earth Environ. 3:477–493. doi:10.1038/s43017-022-00298-5

Preliminary Study on Multispectral Imaging Techniques for Early Pest Detection in Rice Production

Huai-Wen Tsao[1], Yu-Shun Huang[1], Shou-Horng Huang[2], Po-Yu Lai[3], and Ming-Hsin Lai[4,*]

Abstract

Tsao, H. W., Y. S. Huang, S. H. Huang, P. Y. Lai, and M. H. Lai. 2025. Preliminary study on multispectral imaging techniques for early pest detection in rice production. J. Taiwan Agric. Res. 74(3):317–329.

This study focuses on the brown planthopper (*Nilaparvata lugens* (Stål)), a major pest in rice cultivation, and proposes an early-stage pest detection approach that integrates artificial infestation experiments with unmanned aerial vehicle-based multispectral imaging. Treatments with varying insect population densities were simulated, and rice spectral responses were monitored using multispectral imagery collected during the experiment. In this study, the term "early stage" refers to the period between the panicle initiation and heading stages, and corresponds to the typical outbreak period of brown planthopper (BPH) infestations in the second cropping season. Results showed that when the normalized difference vegetation index (NDVI) deviated from the control group by more than the natural variation range (± 1*s*), such deviation could serve as an early-warning signal with the potential to indicate the onset of BPH infestation. The feasibility of using rice plants near field borders as a substitute reference for formal control plots was also evaluated to improve the feasibility for field operation. The proposed approach provides a quantifiable basis for pest monitoring and serves as a potential alternative to traditional manual scouting in the context of smart agriculture.

Key words: Brown planthopper, Insect pest monitoring, Multispectral, Smart agriculture.

Received: April 17, 2025; Accepted: June 16, 2025.
[*] Corresponding author, e-mail: mhlai@tari.gov.tw
[1] Project Assistants, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[2] Associate Research Fellow and Head, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[3] Assistant Research Fellow, Department of Plant Protection, Chiayi Agricultural Experiment Branch, Taiwan Agricultural Research Institute, Chiayi, Taiwan, ROC.
[4] Research Fellow, Crop Science Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):331–343 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0010 研究報告

降低外銷番石榴夏秋季果實罹病率之病害整合管理策略

蔡蕣隍 [1,*]

摘要

蔡蕣隍。2025。降低外銷番石榴夏秋季果實罹病率之病害整合管理策略。台灣農業研究 74(3):331–343。

為減少臺灣番石榴在貯運期的病害，果實採收前的病害管理非常重要。本研究首先調查夏秋季「珍珠拔」番石榴果實病害，黑星病是夏秋季造成番石榴果實腐爛的主要病害，炭疽病、瘡痂病、疫病及其他果腐病則較為次要；至於無病徵葉片、田間落葉及枝條之真菌菌相以擬莖點黴屬 (*Phomopsis* spp.) 分離率最高，可達 100%，另外也有分離到黑星病菌屬 (*Phyllosticta* spp.) 等果實上常見病原，且 84.6% 黑星病菌分離株具有病原性。以動植物防疫檢疫署 (防檢署) 核准之番石榴殺菌劑與市售微生物農藥進行田間果實病害藥效評估，根據試驗結果擬定外銷供果園夏秋季病害整合管理策略 (integrated disease management; IDM)，具體措施從田間衛生開始，先清除果園內殘枝落葉，並配合綠色資材以石灰硫磺合劑或波爾多液噴灑地面。以輸加拿大或美國為例，田間用藥管理建議使用雙方皆核准的藥劑，包括：25% 亞托敏水懸劑、液化澱粉芽孢桿菌 (CL3) 水懸劑或貝萊斯芽孢桿菌 (BF) 可濕性粉劑共同混合施用，每 7–10 d 施用 1 次，連續 3 次，後續應輪用不同作用機制的殺菌劑與微生物資材，可減少病原抗藥性的發生或農藥殘留超標風險。

關鍵詞：外銷番石榴、黑星病、亞托敏、芽孢桿菌、病害整合管理策略。

前言

番石榴 (*Psidium guajava* L.) 為桃金孃科 (Myrtaceae) 番石榴屬 (*Psidium*) 之熱帶果樹，在臺灣各地廣泛種植，近幾年栽培面積多維持在 7,000 多公頃，根據 2023 年農業統計年報 (https://agrstat.coa.gov.tw/sdweb/public/book/Book.aspx)，番石榴總栽培面積達 8,006 ha，年產量約 187,412 Mt，由 2022–2024 年財政部關稅總局海關進出口貿易統計資料顯示，每年平均外銷量 2,039 Mt，故目前臺灣番石榴仍以內銷為主，但國內市場常因供需失調引起價格波動，為穩定國內市場，拓展外銷市場有其必要，農業部在歷經 10 年努力後，終於在 2019 年達成番石榴首輸美國的紀錄，目前最主要外銷之國家 (地區) 分別為加拿大 (64.9%)、香港 (21.7%)、中國大陸 (4.6%)、新加坡 (4.5%) 及美國 (3.6%)。由於成本考量，運輸以海運為主。番石榴果品從採收到市場櫥架販售，所需時間約 30–35 d，在出庫後的回溫販售階段，仍會有病害產生。探究其原因，以番石榴炭疽病為例，國外研究顯示在番石榴花苞及未成熟果實即開始潛伏感染，但直到果實成熟才開始顯現病徵，故田間的病害會影響後續果實的儲藏與櫥架壽命，建議防治應從田間做起 (Guédez & Rodríguez 2021)。由於在夏秋高溫多雨生產的果實品質及良率受病害影響甚鉅，尤其輸美番石榴的低溫檢疫條件 (1℃ 以下持續 17 d 殺滅東方果實蠅、瓜實蠅及南瓜實蠅) 與農藥殘留規定較國內嚴格，習慣於內銷市場為主的栽培用藥管理方式需進行調整，以符合目標市場之規定。

病害整合管理策略 (integrated disease management; IDM) 在多國被證實為有效，結合田間衛生管理、抗病品種選育及生物性與化學性

投稿日期：2025 年 4 月 24 日；接受日期：2025 年 7 月 22 日。
* 通訊作者：tsh@tari.gov.tw
[1] 農業部農業試驗所鳳山熱帶園藝試驗分所植物保護暨園產品加工系助理研究員。臺灣 高雄市。

防治措施，可降低對藥劑的依賴，並提升整體病害防治成效 (El Khoury & Makkouk 2010)。目前國內針對番石榴病害整合管理的研究較為缺乏，國外則有零星之研究報告，針對地下部番石榴根瘤線蟲 (*Meloidogyne enterolobii*)，有較多樣整合管理方法被提出，包括使用土壤添加物、生物防治 (微生物製劑)、化學防治 (殺線蟲劑) 及耕作防治 (輪作) 等，可有效降低番石榴根瘤線蟲罹病嚴重度並增加產量 (Dawabah *et al*. 2019; Sikandar *et al*. 2023; Gondo *et al*. 2025)；對於番石榴地上部病害，部分研究侷限在生體外 (*in vitro*) 測試，例如在巴基斯坦，篩選化學防治藥劑、真菌生物製劑及植物促生根圈細菌 (plant growth promoting rhizobacteria; PGPR) 對番石榴炭疽病菌落生長抑制效果 (Imran-ul-Haq *et al*. 2013)。另針對地上部常見之番石榴果實病害，例如：黑星病，則少有實際整合田間衛生、化學防治及微生物資材於田間測試之研究報告。

本研究針對外銷果園，除了調查夏秋季番石榴果實、葉片及枝條的主要與次要病害種類，並以動植物防疫檢疫署 (防檢署) 核准之番石榴殺菌劑與市售微生物農藥進行田間藥效評估，就前期試驗結果擬定病害整合管理策略 (IDM)，提供農友在田間進行番石榴果實病害防治之參考。

材料與方法

夏秋季番石榴果實主要病害與葉片、枝條菌相調查

為瞭解目前外銷最大宗的「珍珠拔」品種於夏秋季生產時的主要病害種類，於 2022 年 5−10 月間，選定高雄市阿蓮區外銷 (輸美) 供果園 (N22.894207°, E120.315557°)，每個月調查果實病害發生情形，調查方法為果園內劃分成等比例的 5 個區域，每個區域逢機調查 1 株果樹，每株以逢機的方式選取 20 粒成熟果實攜帶回實驗室調查，共調查 100 粒果實。根據 Tsai & Hsieh (2024) 發表之果實黑星病、瘡痂病及炭疽病內轉錄間隔區 (internal transcribed spacer; ITS) 分子鑑定的方法，配合果實病徵、菌落外觀及孢子形態判斷果實病害與病原菌的種類至屬名，並於採後第 0、7 及 14 天記錄感染果實的病原菌屬名，再依照以下公式計算各病害的罹病率：罹病率 (disease incidence, %) = (罹病果數/總調查果數) × 100%。

另外，除了果實病害，植株葉片、落葉以及枝條也列入調查對象，目的是瞭解病原菌在田間的生態棲位 (niche)。在同一個園區以逢機的方式調查 5 株，每個月每株摘取 5 片健康無病徵葉子、落葉 5 片及 5 支新鮮枝條，以相對嚴苛的消毒條件進行組織分離 (Douanla-Meli *et al*. 2013)，培養於馬鈴薯葡萄糖瓊脂 (potato dextrose agar; PDA) 培養基平板，7 d 後觀察所長出之菌落形態，以菌落形態及孢子種類鑑定至屬名，再依照以下公式計算分離率：分離率 (frequency, %) = (分離到該菌之樣本數/總調查樣本數) × 100%。

果實病害藥劑防治效果評估試驗

本研究以防檢署核准之番石榴殺菌劑，針對夏秋季果實之黑星病與次要之炭疽病及瘡痂病進行防治效果評估試驗。參考 Tsai & Hsieh (2024) 藥劑平板篩選的結果中，選取抑菌效果較佳，即藥劑濃度在 100 ppm 時，對黑星病、瘡痂病及炭疽病菌絲生長抑制率皆達 85% 以上之殺菌劑，包括：62.5% 賽普護汰寧水分散性粒劑 (water dispersible granules; WG) (cyprodinil + fludioxonil，臺灣先正達股份有限公司，臺灣臺北市)、40.0% 克熱淨可濕性粉劑 (wettable powder; WP) (iminoctadine tris，日本曹達株式會社，日本東京都)、80.0% 鋅錳乃浦可濕性粉劑 (WP) (mancozeb，惠光股份有限公司，臺灣臺南市)、39.5% 扶吉胺水懸劑 (suspension concentrate; SC) (fluazinam，臺灣石原產業股份有限公司，臺灣臺北市) 及 25.9% 得克利水基乳劑 (emulsion, oil in water; EW) (tebuconazole，臺灣拜耳股份有限公司，臺灣臺北市)；除上述藥劑外，美方核准之殺菌劑 25.0% 亞托敏水懸劑 (SC) (azoxystrobin，臺灣先正達股份有限公司，臺灣臺北市)，及農民常使用之 53.0% 腐絕快得寧可濕性粉劑 (WP) (thiabendazole + oxine copper，光華化學股份有限公司，臺灣新竹市) 也列入田間防治試驗評估。本研究於農業部農業試驗所鳳山

熱帶園藝試驗分所 (簡稱鳳山分所) 之番石榴試驗田 (N22.641313°, E120.363052°)，田間採自然發病，測試前述 7 種藥劑於田間之防治效果，藥劑稀釋倍數如下：賽普護汰寧稀釋 1,500 倍、克熱淨稀釋 1,500 倍、鋅錳乃浦稀釋 400 倍、扶吉胺稀釋 2,000 倍、得克利稀釋 1,500 倍、亞托敏稀釋 2,000 倍及腐絕快得寧稀釋 1,200 倍。以軟質塑膠杯容器盛裝稀釋後藥劑，將夏秋季番石榴小果 (果徑約 1.5−2 cm) 完整浸沒入藥水，每粒浸泡 5 s，目的是使藥劑均勻覆蓋果實表面，無施藥死角，於 4 株植株隨機標記果實，每藥劑共 20 粒果實，每 7 d 施用 1 次，連續處理 3 次，將處理的果實套袋，約過 53−60 d 陸續採收，於採收後第 0、3、6 及 9 天觀察夏秋季果實上病害罹病率 (%) 與黑星病的平均罹病度，本實驗重複 1 次。罹病率 (%) = (罹病果數/總調查果數) × 100%。罹病級數依據病斑占整體果實面積分為 0 級，果實健康無任何病徵；1 級，果實上病斑面積占 1−5%；2 級，果實上病斑面積占 6−25%；3 級，果實上病斑面積占 26−50%；4 級，果實上病斑面積占 51% 以上，再依照下列公式計算平均罹病度：平均罹病度 = Σ (罹病級數 × 該等級之罹病果數)/總調查果數。

市售微生物資材田間防治試驗評估

為評估市售微生物資材對番石榴果實整體病害及單一黑星病的防治效果，於鳳山分所的番石榴試驗田，採逢機完全區集設計 (randomized complete block design; RCBD) 進行微生物資材防治試驗，田間採自然發病。試驗組別包括液化澱粉芽孢桿菌水懸劑 (SC) (*Bacillus amyloliquifaciens* CL3，興農股份有限公司，臺灣臺中市)、液化澱粉芽孢桿菌可濕性粉劑 (WP) (*B. amyloliquifaciens* YCMA1，百泰生物科技股份有限公司，臺灣新竹市)、蕈狀芽孢桿菌可濕性粉劑 (WP) (*B. mycoides* AGB01，聯發生物科技股份有限公司，臺灣屏東縣)、枯草桿菌水懸劑 (SC) (*B. subtilis* KHY8，嘉農企業股份有限公司，臺灣新竹市)、50% 枯草桿菌可濕性粉劑 (WP) (*B. subtilis* Y1336，百泰生物科技股份有限公司，臺灣新竹市)、貝萊斯芽孢桿菌可濕性粉劑 (*B. velezensis* BF，亞亮生技股份有限公司，臺灣屏東縣) 以及水對照組 (CK_water) 共 7 種處理，每處理 3 重複。藥劑稀釋倍數如下：液化澱粉芽孢桿菌 CL3 稀釋 400 倍、液化澱粉芽孢桿菌 YCMA1 稀釋 600 倍、蕈狀芽孢桿菌 AGB01 稀釋 400 倍、枯草桿菌 KHY8 稀釋 500 倍、枯草桿菌 Y1336 稀釋 500 倍及貝萊斯芽孢桿菌 BF 稀釋 600 倍。自番石榴花謝後 (果徑約 0.6−0.7 cm) 開始施用，於夏季 5−6 月份施用，7−10 d 施藥 1 次，連續施用 4 次後套袋，套袋後約 60−67 d 陸續採收，於採收後第 0、3、6 及 9 天觀察夏秋季果實上病害罹病率 (%) 及黑星病的平均罹病度，每處理總共調查 60 粒果實，依照前述公式計算夏秋季果實罹病率 (%) 及黑星病平均罹病度。

夏秋季番石榴果實病害整合管理策略

根據果實病害藥劑與市售微生物資材田間篩選試驗結果，擬定夏秋季番石榴病害整合管理策略，並於鳳山分所的番石榴試驗田進行田間試驗。設計採逢機完全區集設計 (RCBD)，共 2 處理，每處理 3 重複，試驗組別包括水對照組 (CK_water) 與夏秋季番石榴病害整合管理策略 (IDM)，IDM 為混合 25% 亞托敏水懸劑 (SC) 稀釋 2,000 倍與液化澱粉芽孢桿菌水懸劑 (SC) (*B. amyloliquifaciens* CL3) 稀釋 400 倍，自番石榴花謝後 (果徑約 0.6−0.7cm) 開始施用，於 2023 年 6−7 月份施用，7−10 d 施藥 1 次，連續施用 3 次，配合石灰硫磺稀釋 800 倍噴灑地面，藥劑施用後 1 wk 人工接種分離自試驗田之黑星病菌 (GPFS3R)，將 10 盤於 25℃ (12 h 光照/12 h 黑暗) 培養在 PDA 10 d 已產孢之黑星病菌製作孢子懸浮液，以水稀釋至 10 L (最終濃度 1.16×10^4 孢子/mL)，噴灑試驗植株，待果表乾燥後套袋。而為降低蟲害干擾病害試驗，在 3 次施藥期間使用防檢署核准之藥劑防治蟲害，如使用 20% 亞滅培水溶性粉劑 (water soluble powder; SP) (acetamiprid，億豐農化廠股份有限公司，臺灣臺中市) 與 10% 克凡派水懸劑 (SC) (chlorfenapyr，臺灣巴斯夫股份有限公司，臺灣臺北市) 防治薊馬，另以 11% 百利普芬乳劑 (emulsifiable concentrate; EC) (pyriproxyfen，臺灣庵原農藥股份有限公

司，臺灣臺北市) 防治粉介殼蟲，殺蟲劑於套袋完成後即不再使用。藥劑處理完約53−60 d，果實成熟後採收調查，每處理調查 25 粒果實 (3 重複共 75 粒果實)，記錄果實上罹病級數，依照前述公式計算果實罹病率 (%) 與黑星病平均罹病度。另於果實採收當天每處理組額外取樣本 (每處理組 1 kg 果實) 送至屏東科技大學水產檢驗中心進行農藥殘留檢測，以瞭解實際農藥殘留狀況，俾利作為符合外銷果實規範之參考。

統計分析

防治試驗處理之罹病率 (%)，使用 R 統計 (v4.3.3)，以 Shapiro-Wilk test 檢定常態性，再以變方分析 (analysis of variance; ANOVA) 檢驗組間是否顯著差異 ($\alpha = 0.05$)，結果為顯著時 ($P < 0.05$)，對於果實病害藥劑與市售微生物資材田間篩選試驗，以最小顯著性差異 (least significant difference; LSD) 測驗，在 5% 顯著水準下比較處理間罹病率之差異；夏秋季番石榴果實病害整合管理試驗之罹病率，則以 *t* 檢定 (Student's *t*-test) 比較。

防治試驗處理之黑星病平均罹病度，以無母數統計 (Kruskal-Wallis test) 分析，具顯著差異 ($P < 0.05$) 後進行多重比較 (Dunn's test)，在 5% 顯著水準下比較處理間平均罹病度之差異。

結果

夏秋季番石榴果實主要病害與葉片、枝條菌相調查

夏秋季果實病害在採後第 0 天以黑星病 (*Phyllosticta* spp.) 罹病率最高，2022 年 7−10 月平均罹病率約 17.0%，其次是炭疽病 (*Colletotrichum* spp.) 與瘡痂病 (*Neopestalotiopsis* spp.) 約 1.25% (圖 1A)；後續在室溫觀察同一批果實，採後第 7 天黑星病罹病率在 2022 年 9 月最高達 78.0%，炭疽病及瘡痂病平均約占 2.41% (圖 1B)；採後第 14 天黑星病罹病率在 2022 年 8 月最高達 89.0%，炭疽病及瘡痂病則上升至平均約 8.58% (圖 1C)。其餘病害尚包括疫病 (學名前為 *Phytophthora parasitica* 現修正為 *Phytophthora nicotianae*)、*Phomopsis* spp.、*Lasiodiplodia* spp.、*Neofusicoccum* spp. 及 *Cylindrocladium* spp. 引起的果實腐敗，但這些病害僅零星發生，因此黑星病仍是夏秋季造成番石榴果實腐爛的主要病害，炭疽病、瘡痂病、疫病及其他果腐病則較為次要。

無病徵葉片的真菌菌相，以 *Phomopsis* 屬的分離率最高，2022 年 8 月最高達 92.0%，其次是 *Phyllosticta* 屬，7 月達 60.0%，其餘 *Colletotrichum* 屬、*Neopestalotiopsis* 屬及 *Lasiodiplodia* 屬 2022 年 5−10 月平均約 7.5% (圖 1D)；田間落葉的真菌菌相以 *Phomopsis* 屬分離率最高，2022 年 9 月最高達 96.0%，其次是 *Neopestalotiopsis* 屬，8 月達 48.0%，其餘 *Colletotrichum* 屬、*Lasiodiplodia* 屬及 *Phyllosticta* 屬 2022 年 5−10 月平均約 9.1% (圖 1E)；新鮮枝條的真菌菌相仍以 *Phomopsis* 屬分離率最高，2022 年 8−9 月最高達 100.0%，其次是 *Lasiodiplodia* 屬，6 月達 40.0%，其餘 *Colletotrichum* 屬、*Neopestalotiopsis* 屬及 *Phyllosticta* 屬 2022 年 5−10 月平均約 15.7% (圖 1F)。

果實病害藥劑防治效果評估試驗

本研究評估賽普護汰寧、克熱淨、鋅錳乃浦、扶吉胺、得克利、亞托敏及腐絕快得寧等 7 種藥劑實際於田間之防治效果，結果如圖 2 所示。採收後第 0 天 25% 亞托敏水懸劑對於病害罹病率 (僅 20 ± 8.16%) (圖 2A) 或是黑星病平均罹病度 (僅 0.05 ± 0.05) (圖 2B) 均有甚佳防治效果；於採收後第 9 天，亞托敏無論對於病害罹病率 (80.0 ± 0.0%) (圖 2A) 或是黑星病平均罹病度 (1.15 ± 0.19) (圖 2B) 相較水處理對照組或其他測試殺菌劑均有較佳之病害延緩效果，具顯著差異。因此後續選取此藥劑作為生產夏秋季果實主要化學殺菌劑，並於生產冬春季果實適當輪用其他效果較佳之殺菌劑、微生物製劑或有機資材，降低抗藥性產生之風險。

市售微生物資材田間防治試驗評估

本研究評估液化澱粉芽孢桿菌 (*B. amyloliquifaciens* CL3)、液化澱粉芽孢桿菌 (*B. amyloliquifaciens* YCMA1)、蕈狀芽孢桿菌

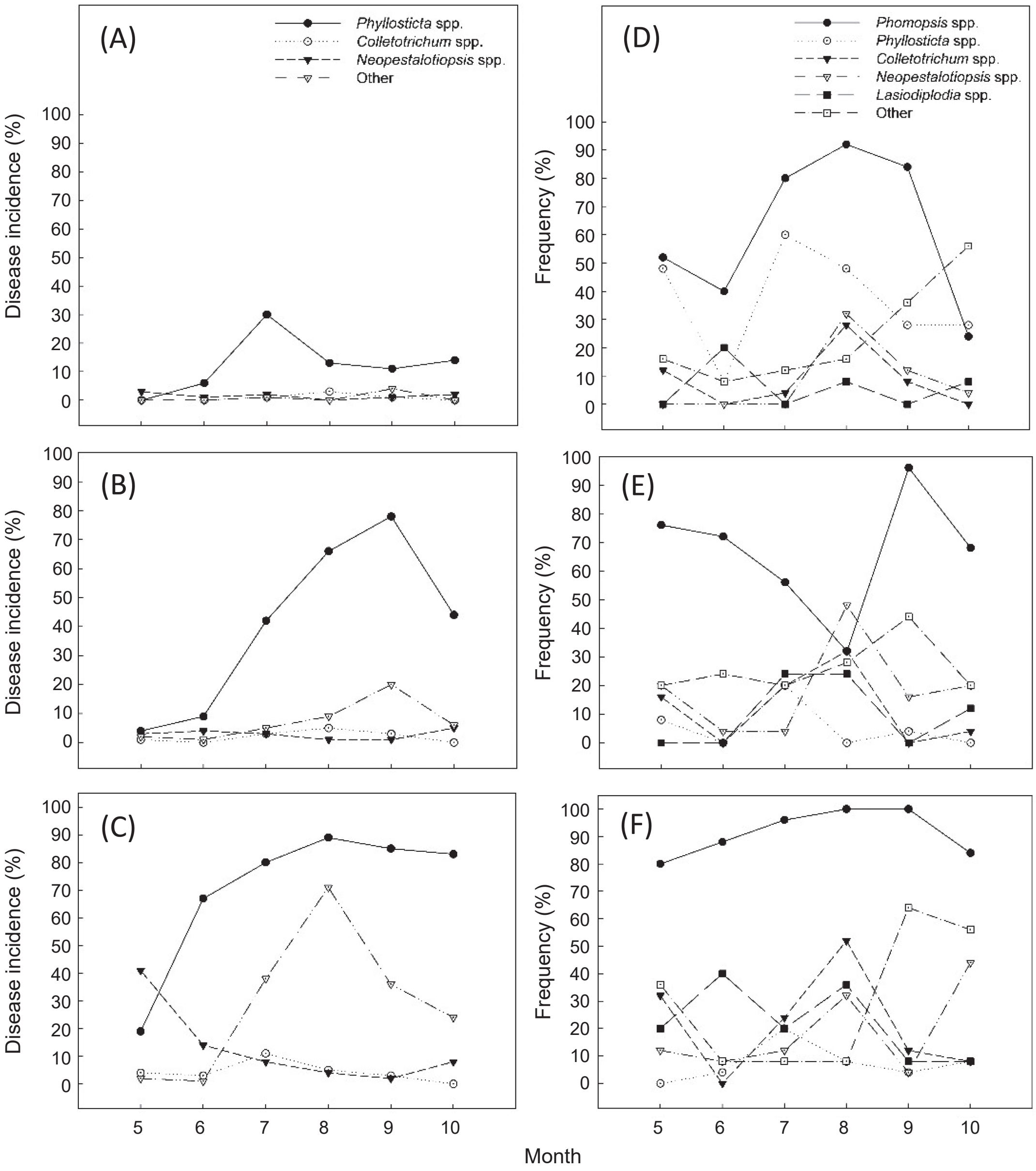

圖 1. 2022 年 5–10 月阿蓮區輸美供果園果實採後第 (A) 0、(B) 7 及 (C) 14 天之病害罹病率與 (D) 健康葉片、(E) 落葉及 (F) 新鮮枝條上之真菌相與分離率。

Fig. 1. The disease incidence of guava fruits detected at (A) 0, (B) 7, and (C) 14 d post-harvesting and the fungal isolation rates isolated from (D) symptomless leaves, (E) leaf litter, and (F) fresh branches from an orchard in Alian District between May and October, 2022.

(*B. mycoides* AGB01)、枯草桿菌 (*B. subtilis* KHY8)、枯草桿菌 (*B. subtilis* Y1336) 及貝萊斯芽孢桿菌 (*B. velezensis* BF) 等 6 種微生物製劑對果實病害及黑星病的防治效果，結果如圖 3 所示。液化澱粉芽孢桿菌 (CL3) 與貝萊斯芽孢桿菌 (BF) 對番石榴果實病害或黑星病的防治效果相較對照組為佳，具顯著差異，採後第 0 天的果實病害罹病率 (%) 分別為 40.0

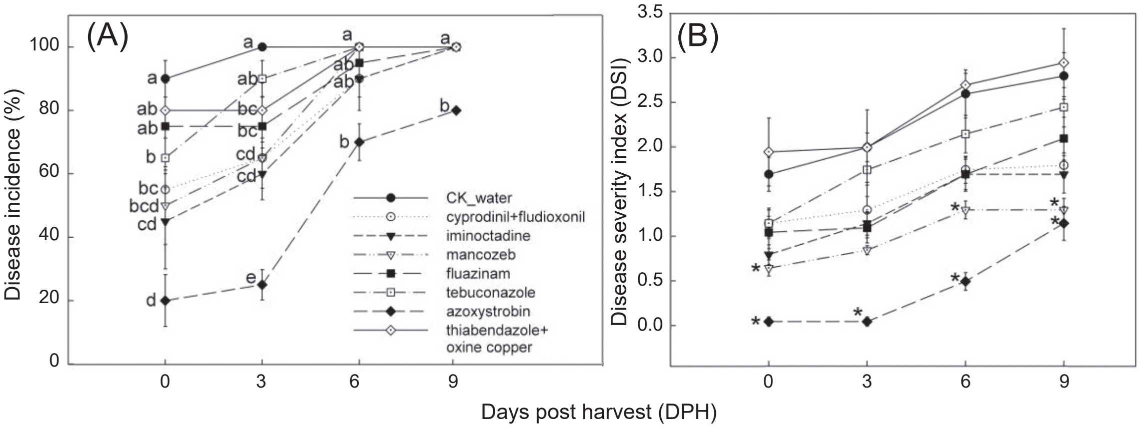

圖 2. 評估賽普護汰寧、克熱淨、鋅錳乃浦、扶吉胺、得克利、亞托敏及腐絕快得寧處理幼果對採收後第 0、3、6、9 天夏秋季番石榴果實病害及黑星病的防治效果，並以水處理作為對照組。(A) 折線圖為病害平均罹病率 (%)，誤差線代表標準誤差，以最小顯著差異法 (least significant difference; LSD) 進行統計檢定，在同一觀察時間點內，各處理之間若英文符號不同者表示具有顯著差異 ($P < 0.05$)；(B) 折線圖為黑星病之平均罹病度，誤差線代表標準誤差，以無母數統計 (Kruskal-Wallis test) 分析後進行多重比較 (Dunn's test)，星號 (*) 代表在 5% 顯著水準下具顯著差異。

Fig. 2. Evaluating the control efficacy of tested fungicides including cyprodinil + fludioxonil, imonoctadine tris, mancozeb, fluazinam, tebuconazole, azoxystrobin, and thiabendazole + oxine copper on the disease incidence of guava fruits and black spot severity in guava fruits detected at 0, 3, 6, and 9 DPH during the summer and autumn seasons, with water treatment (CK) serving as the control. (A) Line graphs represent mean disease incidence within each treatment, and error bars indicate standard error. Means within each observation day followed by different letters indicate significant differences according to the least significant difference (LSD) test ($P < 0.05$); (B) Line graphs represent the mean severity of black spot disease for each treatment, with error bars indicating the standard error. Data were analyzed using the non-parametric Kruskal-Wallis test; multiple comparisons were conducted using Dunn's test, with asterisks (*) indicating significant differences at the 5% significance level.

± 0.0% 與 45.0 ± 4.3% (圖 3A)，黑星病平均罹病度分別為 0.35 ± 0.09 與 0.7 ± 0.06 (圖 3B)；另可觀察到液化澱粉芽孢桿菌 (CL3) 與貝萊斯芽孢桿菌 (BF) 僅延緩儲藏期黑星病發展，對照組採後第 9 天的果實黑星病平均罹病度為 2.7 ± 0.26 (圖 3B)，而液化澱粉芽孢桿菌 (CL3) 與貝萊斯芽孢桿菌 (BF) 採後第 9 天的果實黑星病平均罹病度分別為 1.75 ± 0.1 與 1.8 ± 0.14 (圖 3B)，但兩者對儲藏期病害罹病率的降低無顯著差異 (圖 3A)。根據此結果，後續選取液化澱粉芽孢桿菌 (CL3) 作為病害整合管理策略防治藥劑，貝萊斯芽孢桿菌 (BF) 則可候選作為輪用之微生物製劑。

夏秋季番石榴果實病害整合管理策略

據交通部中央氣象局鳳山分所的氣象監測站累積雨量與平均溫度資料顯示，2023 年 6–9 月累積雨量達 1,641.5 mm，平均溫度 28.5℃ (圖 4)，為適合黑星病發病的環境。根據果實病害藥劑與市售微生物資材田間篩選試驗結果，本研究擬定夏秋季番石榴果實病害整合管理策略 (IDM)，即混合亞托敏與液化澱粉芽孢桿菌 (CL3)，依照核准稀釋倍數連續施用 3 次，配合石灰硫磺稀釋 800 倍噴灑地面。結果如圖 5 所示。IDM 處理之果實採後 0 d 的罹病率與黑星病平均罹病度分別為 35.0 ± 7.6% 與 0.6 ± 0.03，對照組罹病率與黑星病平均罹病度分別為 63.3 ± 3.3% 與 1.37 ± 0.06，在 95% 信賴區間下達顯著，顯示該策略可以有效降低夏秋季番石榴果實病害罹病率與黑星病平均罹病度；IDM 處理之果實採後 9 d 的罹病率與黑星病平均罹病度分別為 78.3 ± 1.67% 與 1.52 ± 0.16，對照組罹病率與黑星病平均罹病度分別為 95.0 ± 2.89% 與 2.58 ± 0.14，在 95% 信賴區間下達顯著，顯示該策略可以有效延緩夏秋季番石榴果實儲藏期病害的罹病率與黑星病平均罹病

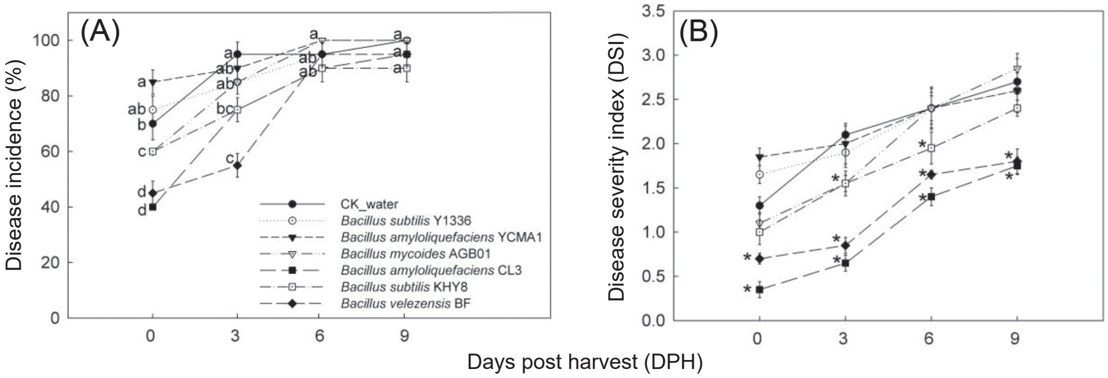

圖 3. 評估枯草桿菌 Y1336、液化澱粉芽孢桿菌 YCMA1、蕈狀芽孢桿菌 AGB01、液化澱粉芽孢桿菌 CL3、枯草桿菌 KHY8 及貝萊斯芽孢桿菌 BF 對採收後第 0、3、6、9 天夏秋季番石榴果實病害及黑星病的防治效果，並以水處理作為對照組。圖中評估方法與圖 2 相同。(A) 為病害平均罹病率 (%)；(B) 為黑星病平均罹病度，誤差線表示標準誤差，統計分析分別採用最小顯著差異法 (least significant difference; LSD) 與 Kruskal-Wallis + Dunn's test，顯著性標示方式同圖 2。

Fig. 3. Evaluating the control efficacy of tested microbial fungicides including *Bacillus subtilis* Y1336, *B. amyloliquefaciens* YCMA1, *B. mycoides* AGB01, *B. amyloliquefaciens* CL3, *B. subtilis* KHY8, and *B. velezensis* BF on the disease incidence of guava fruits and black spot severity in guava fruits detected at 0, 3, 6, and 9 DPH during the summer and autumn seasons, with water treatment (CK) as the control. The methods of evaluation and statistical analyses are the same as described in Fig. 2. (A) Mean disease incidence; (B) Mean severity of black spot disease. Error bars indicate standard error; significance testing and annotations follow the same criteria as in Fig. 2.

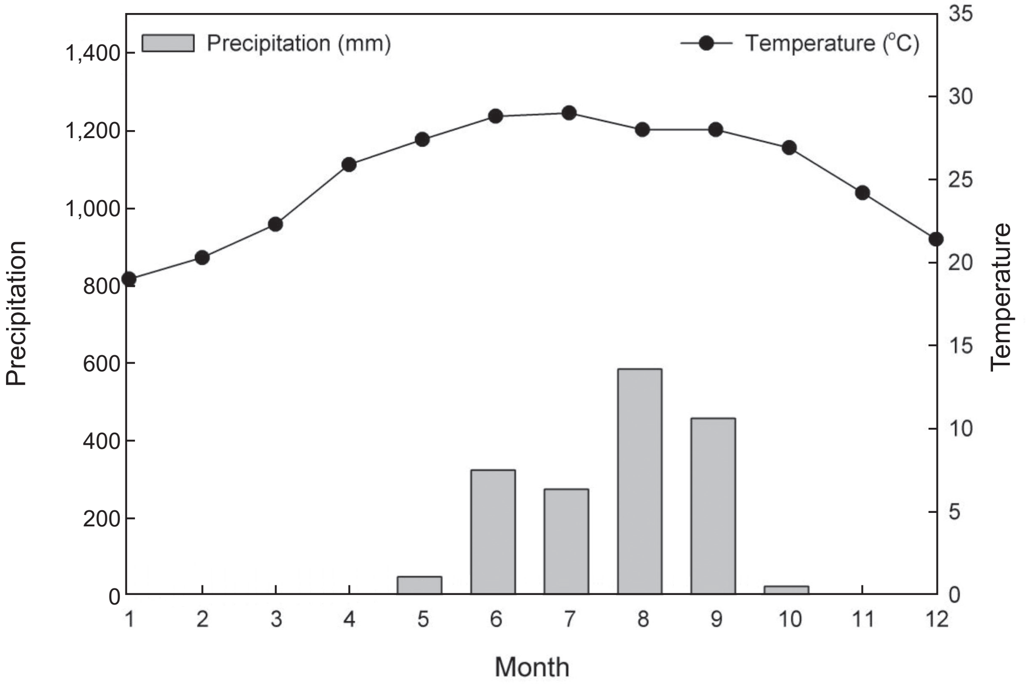

圖 4. 2023 年 1–12 月鳳山熱帶園藝試驗分所氣象監測站平均溫度與累積雨量分布。

Fig. 4. Monthly average temperature and cumulative precipitation measured at the Fengshan Tropical Horticultural Experiment Branch in 2023.

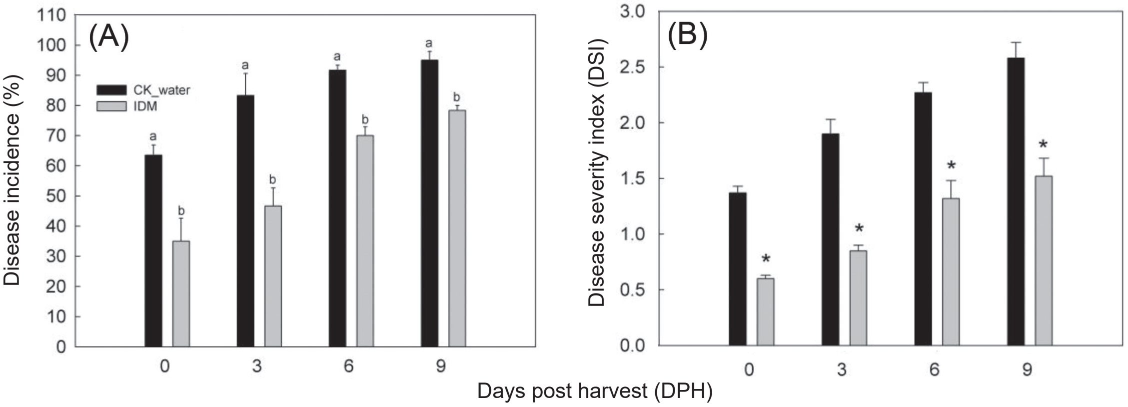

圖 5. 測試病害整合管理策略 (IDM) 對採收後第 0、3、6、9 天夏秋季果實病害及黑星病的防治效果，並以水處理作為對照組。(A) 長條圖為病害平均罹病率 (%)，誤差線代表標準誤差，以 *t* 檢定 (Student's *t*-test) 進行統計檢定，在同一觀察時間點內，各處理之間若英文符號不同者表示具有顯著差異 ($P < 0.05$)；(B) 長條圖為黑星病之平均罹病度，誤差線代表標準誤差，以無母數統計 (Kruskal-Wallis test) 分析後進行多重比較 (Dunn's test)，星號 (*) 代表在 5% 顯著水準下具顯著差異。

Fig. 5. Evaluating the control efficacy of integrated disease management (IDM) on the disease incidence of guava fruits and black spot severity in guava fruits detected at 0, 3, 6, and 9 DPH during the summer and autumn seasons, with water treatment (CK) serving as the control. (A) Bars represent mean disease incidence within each treatment, and error bars indicate standard error. Means within each observation day followed by different letters indicate significant differences according to the Student's *t*-test ($P < 0.05$); (B) Bars represent the mean severity of black spot disease for each treatment, with error bars indicating the standard error. Data were analyzed using the non-parametric Kruskal-Wallis test; multiple comparisons were conducted using Dunn's test, with an asterisk (*) indicating significant differences at the 5% significance level.

度。並且農藥殘留檢測報告結果顯示使用之藥劑皆未檢出，符合外銷之需求。

討論

根據《臺灣植物病害名彙》(第五版) 有紀錄的番石榴病害 39 種，真菌與類真菌病害 15 種，線蟲 24 種 (Tzean *et al*. 2019)。Lin *et al*. (2005) 撰寫之臺灣中南部田間番石榴病害，果實的主要病害有黑星病 (*Phyllosticta psidiicola*)、瘡痂病 (*Pestalotiopsis psidii*)、炭疽病 (*Colletotrichum gloeosporioides*)、疫病 (*Phytophthora parasitica*) 及果腐病 (包括：*Phomopsis* spp., *Botryodiplodia* spp., *Rhizoctonia* sp. 及 *Botrytis cinerea*) 等真菌與類真菌性病害，由於資料已近 20 年未更新，有必要重啟田間調查，以確認主要病害種類或是否有新病害發生。故本研究於夏秋季多雨季節調查「珍珠拔」主要果實病害，少數果腐病種類與 Lin *et al*. (2005) 調查報告略有不同，如未採集到少數之果腐病菌如 *Rhizoctonia* sp. 與 *Botrytis cinerea*，但觀察到 *Cylindrocladium* 屬引起的果實腐敗，至於主要病害種類仍相同，故後續田間的防治重點仍在黑星病。

本研究另以較嚴苛消毒方式分離培養無病徵葉片、田間落葉及新鮮枝條內部的真菌，目的是欲瞭解除果實以外，其他組織內部的菌相是否與果實病害相關。結果顯示無病徵葉片病原真菌之分離率，依高低順序為 *Phomopsis* 屬、*Phyllosticta* 屬、*Colletotrichum* 屬、*Neopestalotiopsis* 屬及 *Lasiodiplodia* 屬，這些真菌種類為重要之植物病原真菌，皆會感染果實。國外文獻亦顯示番石榴無病徵葉片內可分離到多達 28 個屬之真菌，其中包括 *Xylaria* 屬、*Phomopsis* 屬、*Aspergillus* 屬及 *Phyllosticta* 屬等 (Tchamgoue *et al*. 2020)；此外，草莓番石榴 (*Psidium cattleianum*) 葉片內亦分離培養出多達 25 個屬之真菌，包括 *Alternaria* 屬、*Aspergillus* 屬、*Cladosporium* 屬及 *Colletotrichum* 屬等 (André *et al*. 2024)，顯示葉

片內真菌族群的生物多樣性，雖然這些真菌種類與本研究分離種類有些不同，但部分為常見之植物病原真菌。本研究亦嘗試分離田間落葉與新鮮枝條內部的真菌，值得留意的是，皆以 *Phomopsis* 屬分離率 (%) 最高，*Phomopsis* 屬歸類在真菌界子囊菌門 (Ascomycota) 腔孢菌綱 (Coelomycetes)，其有性世代為間座殼屬 (*Diaporthe*)，根據 Mycobank (http://www.mycobank.org/)，目前 *Phomopsis* 屬有 1,057 個種，*Diaporthe* 屬則有 1,372 種 (上網日期 2025/3/18)，該屬為腐生菌 (saprobes)、內生真菌 (endophytes) 或重要植物病原真菌 (Webber & Gibbs 1984; Udayanga *et al*. 2011)，可造成多種果樹之果實病害，例如藍莓與蔓越莓之枝枯病 (*Phomopsis vaccinii* 與其他 *Phomopsis* sp.) (Farr *et al*. 2002)、葡萄枝枯病 (*Phomopsis viticola*) (Merrin *et al*. 1995)、芒果蒂腐病 (*Phomopsis mangiferae*) (Ko *et al*. 2009)、酪梨蒂腐病 (*Phomopsis* sp.) (Twizeyimana *et al*. 2013) 及番石榴臍腐病 (*Phomopsis* sp.) (Rai 1956) 等。本研究有分離到零星的 *Phomopsis* 病害在儲藏期間 (果實採後 7 d 與 14 d) 逐漸出現在觀察的果實上，特別是在果頂的位置，推測該菌在田間以落葉及枝條作為初始與二次感染源，在雨季時藉著風雨傳播孢子，潛伏感染尚未套袋小果，於採收後櫥架期間才出現病徵。另外無病徵葉片、田間落葉及新鮮枝條皆有分離到果實上常見之病原真菌，例如 *Phyllosticta* 屬、*Neopestalotiopsis* 屬、*Colletotrichum* 屬及 *Lasiodiplodia* 屬，筆者實際接種從無病徵葉片分離的真菌，以黑星病菌 (*Phyllosticta* spp.) 為例，84.6% 黑星病菌分離株具有病原性，顯示這些潛伏在無病徵葉片的黑星病菌是重要的感染源 (Tsai & Hsieh 2024)，本研究雖未證實田間之黑星病分生孢子作為感染源，但依據 Spósito *et al*. (2007) 研究結果推論，柑橘之黑星病菌 (*Phyllosticta citricarpa*) 可從落葉中主動釋放子囊孢子，藉由風做短與長距離傳播，故田區裡面或周圍的果樹以及其他寄主皆可提供黑星病菌存活。因此，若要提高整體防治效率，降低果實病害的發生，落實田間衛生管理是重要的前提。清園並配合綠色資材，例如石灰硫磺合劑或波爾多液噴灑地面，並清除殘枝落葉，本操作可顯著降低田間病原菌整體密度，降低因病害引起的損失 (Fischer *et al*. 2017)。

因應番石榴外銷，其用藥管理方式須以不同目標市場為依歸，以輸美番石榴為例，針對黑星病，臺灣與美國皆核准之藥劑僅有賽普護汰寧；針對炭疽病，雙方皆核准之藥劑則有亞托敏與賽普護汰寧，其餘病害卻無相對應之防治藥劑，顯示番石榴若要輸美，可用之化學殺菌藥劑種類不多，在此情形下，就必須要針對主要病害選出數種國外准許使用且抑菌力強的藥劑供輪替使用。根據前期實驗室平板藥劑篩選結果，賽普護汰寧、扶吉胺、得克利、克熱淨及鋅錳乃浦等藥劑濃度在 100 ppm 時，對黑星病、瘡痂病及炭疽病菌絲生長抑制率皆達 85% 以上 (Tsai & Hsieh 2024)。除了上述藥劑，本研究另外測試亞托敏與腐絕快得寧實際於田間之防治效果，值得留意的是，前期試驗在生體外測試亞托敏對於番石榴黑星病菌 (GPYC11)、瘡痂病菌 (PPYC3) 及炭疽病菌 (SYCo3) 菌絲生長抑制效果皆不佳 (Tsai & Hsieh 2024)，然而本研究實際將亞托敏施用於田間時，卻可有效降低果實病害或黑星病之罹病率，Everett *et al*. (2005) 對於酪梨果腐病之研究也呼應此結果；相反地，扶吉胺在生體外 (*in vitro*) 測試對於菌絲生長抑制效果十分優異，但在田間試驗表現不佳 (Everett *et al*. 2005)。本研究也觀察到此現象，推測可能原因是由於殺菌劑在田間測試的效果會受到藥劑在葉片與果實表面的滯留時間 (Gent *et al*. 2003) 以及藥劑對 UV 穩定性 (Boudina *et al*. 2003) 的影響，另外 Everett & Neilson (1996) 的研究證實生體外測試的結果可能會排除那些不值得進一步試驗的殺菌劑，因此影響殺菌劑藥效的關鍵不是單純只考量實驗室內篩選而已，還必須考慮殺菌劑實際施用時環境因子之交互作用。

本研究田間果實病害藥劑試驗所評估較佳防治效果的亞托敏為史托比類 (strobilurins) 藥劑，最先發現之主成分為嗜毬果傘素 A (strobilurin A)，分離自蕈菇嗜毬果傘菌 (*Strobilurus tenacellus* 21602) (Anke *et al*. 1977)，後續發現類似抗生物質如奧德蘑素 A (oudemansin A)，因為這些抗生物質容易因光

不穩定導致在溫室試驗效果不佳，經過驗證後發現帶有 stilbene 官能基之 β-methoxyacrylate 為真正發揮毒性作用的結構，後續便以人工合成改進該化學結構，最後篩選出亞托敏成為第一個商品，其殺菌作用機制為抑制粒線體內膜上複合體 III (Complex III) cyt b 與 cyt c_1 間泛醇 (ubiquinol) 氧化位點之電子傳遞，導致呼吸作用中斷無法產生腺苷三磷酸 (adenosine triphosphate; ATP) 而達到殺菌效果 (Becker *et al*. 1981; Von Jagow *et al*. 1986; Brandt *et al*. 1988)，故亞托敏為單點作用機制藥劑，抗藥性屬於高度風險，需進行抗藥性管理。本研究篩選市面上常見之商品化有益微生物，結果顯示液化澱粉芽孢桿菌 (CL3) 與貝萊斯芽孢桿菌 (BF) 對果實病害與黑星病有較佳的防治效果。芽孢桿菌的作用機制多樣，包括促進植物生長、產生具有抗生活性之二次代謝物以及誘導抗病 (Fira *et al*. 2018; Kiesewalter *et al*. 2021)，這些優勢讓芽孢桿菌類製劑可以同時防治多種真菌或細菌病原，類似多位點作用機制的化學殺菌劑，很適合作為殺菌劑抗藥性管理的工具 (Stein 2005)。近期國外許多研究顯示，芽孢桿菌 (*Bacillus* spp.) 可以搭配單點作用機制之系統性殺菌劑共同施用，例如枯草桿菌 (*B. subtilis* BIOUFLA2) 搭配環克座 (cyproconazole) 與亞托敏 (azoxystrobin)，相較單獨施用化學殺菌劑可兼具產量與防治玉米穗腐病 (*Fusarium verticillioides*) (Guimarães *et al*. 2021)；利用枯草桿菌 (*B. subtilis* BV02) 搭配系統性殺菌劑可顯著降低大豆亞洲銹病 (*Phakopsora pachyrhizi*) 的罹病度 (Santos *et al*. 2022)，故微生物資材搭配系統性殺菌劑共同施用，可兼顧作物品質、提高收成良率、降低殺菌劑單劑使用之抗藥性風險以及過度依賴治療性用藥。

目前臺灣番石榴外銷的國家用藥規範較國內相對嚴格，防治策略必須嚴格遵循目標市場共同認可的農藥使用規範，因此在選擇防治藥劑時，需同時考量合法性與有效性，故本研究特別針對外銷番石榴提出夏秋季果實病害整合管理策略。由於臺灣中南部夏秋季多雨，調查結果顯示該季節黑星病是造成番石榴果實腐爛的主要病原，若要進行用藥管理，本研究的防治策略建議使用目標市場 (例如加拿大與美國) 皆核准的藥劑，如亞托敏，並與微生物資材如液化澱粉芽孢桿菌 (CL3) 或貝萊斯芽孢桿菌 (BF) 共同混合施用，每次施用期間間隔 7–10 d，至少要連續使用 3 次，後續透過適當的藥劑輪用，例如賽普護汰寧等殺菌劑與微生物資材共同使用，可減少病原抗藥性的發生並且降低被檢出違規用藥或藥劑殘留超標風險。有一點須注意的是，鋅錳乃浦在本研究對於田間果實黑星病防治效果僅次於亞托敏，但過去研究指出芽孢桿菌類製劑與鋅錳乃浦不相容，故不建議鋅錳乃浦與芽孢桿菌製劑共同混合施用 (Santos *et al*. 2022)。此外，田間管理中的清園措施也不可忽視，配合綠色資材例如石灰硫磺合劑或波爾多液噴灑地面，有助於減少病原菌在田間的密度，從而提高防治效果，並且果農的栽培管理也最重要，有助於樹體維持強健，降低病害發生機會。

總體而言，本研究提供了一套綜合防治方案，將有助於提高夏秋季番石榴果實的病害防治效率，降低病害的罹病率與黑星病平均罹病度，並且確保符合國際市場的出口標準。因此，整合化學與生物防治手段，遵循農藥使用規範，以及配合適當的田間管理措施是有效管理番石榴病害的關鍵。

引用文獻

André, B. L. O., Q. V. Montoya, M. J. S. Martiarena, and A. Rodrigues. 2024. Culture-dependent methods reveal the diversity of endophytic fungi of *Psidium cattleianum* leaves (Myrtales: Myrtaceae). Braz. J. Microbiol. 55:3425–3436. doi:10.1007/s42770-024-01481-0

Anke, T., F. Oberwinkler, W. Steglich, and G. Schramm. 1977. The strobilurins-new antifungal antibiotics from the basidiomycete *Strobilurus tenacellus*. J. Antibiot. 30:806–810. doi:10.7164/antibiotics.30.806

Becker, W. F., G. Von Jagow, T. Anke, and W. Steglich. 1981. Oudemansin, strobilurin A, strobilurin B and myxothiazol: New inhibitors of the bc_1 segment of the respiratory chain with an E-β-methoxyacrylate system as common structural element. FEBS Lett. 132:329–333. doi:10.1016/0014-5793(81)81190-8

Boudina, A., C. Emmelin, A. Baaliouamer, M. F. Grenier-Loustalot, and J. M. Chovelon. 2003. Photochemical behaviour of carbendazim in aqueous solution. Chemosphere 50:649−655. doi:10.1016/S0045-6535(02)00620-3

Brandt, U., H. Schägger, and G. von Jagow. 1988. Characterisation of binding of the methoxyacrylate inhibitors to mitochondrial cytochrome *c* reductase. Eur. J. Biochem. 173:499−506. doi:10.1111/j.1432-1033.1988.tb14026.x

Dawabah, A. A. M., F. A. Al-Yahya, and H. A. Lafi. 2019. Integrated management of plant-parasitic nematodes on guava and fig trees under tropical field conditions. Egypt. J. Biol. Pest Control. 29:29. doi:10.1186/s41938-019-0133-9

Douanla-Meli, C., E. Langer, and F. T. Mouafo. 2013. Fungal endophyte diversity and community patterns in healthy and yellowing leaves of *Citrus limon*. Fungal Ecol. 6:212−222. doi:10.1016/j.funeco.2013.01.004

El Khoury, W. and K. Makkouk. 2010. Integrated plant disease management in developing countries. J. Plant Pathol. 92:S4.35–S4.42.

Everett, K. R. and H. F. Neilson. 1996. Evaluation of fungicides for control of *Alternaria* leaf spot of *Pseudopanax*. N. Z. J. Crop Hortic. Sci. 24:267−272. doi:10.1080/01140671.1996.9513961

Everett, K. R., S. G. Owen, and J. G. M. Cutting. 2005. Testing efficacy of fungicides against postharvest pathogens of avocado (*Persea americana* cv Hass). N. Z. Plant Prot. 58:89−95. doi:10.30843/nzpp.2005.58.4260

Farr, D. F., L. A. Castlebury, and A. Y. Rossman. 2002. Morphological and molecular characterization of *Phomopsis vaccinii* and additional isolates of *Phomopsis* from blueberry and cranberry in the eastern United States. Mycologia 94:494−504. doi:10.2307/3761783

Fira, D., I. Dimkić, T. Berić, J. Lozo, and S. Stanković. 2018. Biological control of plant pathogens by *Bacillus* species. J. Biotechnol. 285:44−55. doi:10.1016/j.jbiotec.2018.07.044

Fischer, I. H., A. R. Soares-Colletti, M. C. de Arruda Palharini, M. C. M. Parisi, and L. Amorim. 2017. Temporal progress and spatial patterns of quiescent diseases in guava influenced by sanitation practices. Sci. Agric. 74:68−76. doi:10.1590/1678-992x-2015-0425

Gent, D. H., H. F. Schwartz, and S. J. Nissen. 2003. Effect of commercial adjuvants on vegetable crop fungicide coverage, absorption, and efficacy. Plant Dis. 87:591−597. doi:10.1094/PDIS.2003.87.5.591

Gondo, I. R. G., M. R. da Rocha, C. Y. Gondo, K. dos Santos Alves, B. B. dos Anjos, S. de Paiva Caetano Bucker Moraes, ... W. B. Moraes. 2025. Temporal dynamics of guava root-knot nematode under the effect of different management methods. Crop Prot. 188:107012. doi:10.1016/j.cropro.2024.107012

Guédez, C. and D. Rodríguez. 2021. Quiescent infections by *Colletotrichum gloeosporioides* in the different development stages of the guava fruit (*Psidium guajava* L.) in two production seasons. Rev. Fac. Agron. (LUZ) 38:751−770. doi:10.47280/RevFacAgron(LUZ).v38.n4.01

Guimarães, R. A., E. Zanotto, P. E. P. Perrony, L. A. S. Zanotto, L. J. da Silva, J. da Cruz Machado, ... F. H. V. de Medeiros. 2021. Integrating a chemical fungicide and *Bacillus subtilis* BIOUFLA2 ensures leaf protection and reduces ear rot (*Fusarium verticillioides*) and fumonisin content in maize. J. Phytopathol. 169:139−148. doi:10.1111/jph.12968

Imran-ul-Haq, M. Sajjad, S. A. Khan, M. J. Jaskani, and Z. Ullah. 2013. Occurrence of guava anthracnose in Punjab (Pakistan) and its integrated management. Pak. J Agri. Sci. 50:707–710.

Kiesewalter, H. T., C. N. Lozano-Andrade, M. Wibowo, M. L. Strube, G. Maróti, D. Snyder, ... Á. T. Kovács. 2021. Genomic and chemical diversity of *Bacillus subtilis* secondary metabolites against plant pathogenic fungi. mSystems 6:e00770-20. doi:10.1128/mSystems.00770-20

Ko, Y., C. W. Liu, C. Y. Chen, S. Maruthasalam, and C. H. Lin. 2009. First report of stem-end rot of mango caused by *Phomopsis mangiferae* in Taiwan. Plant Dis. 93:764. doi:10.1094/PDIS-93-7-0764A

Lin, C. C., C. S. Lai, and S. F. Tsai. 2005. Guava black spot disease. p.75−78. *in*: Illustrated Series of Plant Protection 15- Guava Protection. (Lin, C. C., C. I. Yuan, S. P. Chen, J. S. Hwang, H. C. Wen, W. S. Cai, eds.) Bureau of Animal and Plant Health Inspection and Quarantine, Council of Agriculture, Executive Yuan. Taipei, Taiwan. 205 pp. (in Chinese)

Merrin, S. J., N. G. Nair, and J. Tarran. 1995. Variation in *Phomopsis* recorded on grapevine in Australia and its taxonomic and biological implications. Australas. Plant Pathol. 24:44−56. doi:10.1071/app9950044

Rai, J. N. 1956. Stylar end rot of the guava fruit (*Psidium guajava* linn). Proc. Indian Acad. Sci. 43:55−61. doi:10.1007/BF03050219

Santos, F. M., L. S. Viera, D. P. Camargo, M. F. Muniz, I. F. Costa, J. V. Guedes, ... J. C. Silva. 2022. Integrating a *Bacillus*-based product with fungicides by foliar application to protect soybean: A sustainable

approach to avoid exclusive use of chemicals. Pest Manag. Sci. 78:4832−4840. doi:10.1002/ps.7104

Sikandar, A., L. Jia, H. Wu, and S. Yang. 2023. *Meloidogyne enterolobii* risk to agriculture, its present status and future prospective for management. Front. Plant Sci. 13:1093657. doi:10.3389/fpls.2022.1093657

Spósito, M. B., L. Amorim, P. J. Ribeiro Jr., R. B. Bassanezi, and E. T. Krainski. 2007. Spatial pattern of trees affected by black spot in citrus groves in Brazil. Plant Dis. 91:36−40. doi:10.1094/pd-91-0036

Stein, T. 2005. *Bacillus subtilis* antibiotics: Structures, syntheses and specific functions. Mol Microbiol. 56:845−857. doi:10.1111/j.1365-2958.2005.04587.x

Tchamgoue, E. N., S. A. Y. Fanche, B. L. Ndjakou, F. Matei, and M. A. Nyegue. 2020. Diversity of endophytic fungi of *Psidium guajava* (Myrtaceae) and their antagonistic activity against two banana pathogens. J. Adv. Microbiol. 20:86−101. doi:10.9734/jamb/2020/v20i1130304

Tsai, S. H. and H. Y. Hsieh. 2024. Investigation of guava fruit diseases and fungicide sensitivity. p.29−36. *in*: Proceedings of the Symposium on Research Results of FTHES, TARI. January 11, 2024. Kaohsiung, Taiwan. Taiwan Agric. Res. Inst. Pub. No. 241. Taichung, Taiwan. (in Chinese with English abstract)

Twizeyimana, M., H. Förster, V. McDonald, D. H. Wang, J. E. Adaskaveg, and A. Eskalen. 2013. Identification and pathogenicity of fungal pathogens associated with stem-end rot of avocado in California. Plant Dis. 97:1580−1584. doi:10.1094/PDIS-03-13-0230-RE

Tzean, S. S., K. C. Tzeng, C. A. Chang, T. T. Tsay, and H. F. Yen. (eds.) 2019. List of Plant Diseases in Taiwan. 5th ed. Taiwan Phytopathology Society. Taichung, Taiwan. 329 pp. (in Chinese)

Udayanga, D., X. Liu, E. H. C. McKenzie, E. Chukeatirote, A. H. A. Bahkali, and K. D. Hyde. 2011. The genus *Phomopsis*: Biology, applications, species concepts and names of common phytopathogens. Fungal Divers. 50:189−225. doi:10.1007/s13225-011-0126-9

Von Jagow, G., G. W. Gribble, and B. L. Trumpower. 1986. Mucidin and strobilurin A are identical and inhibit electron transfer in the cytochrome bc1 complex of the mitochondrial respiratory chain at the same site as myxothiazol. Biochemistry 25:775−780. doi:10.1021/bi00352a006

Webber, J. F. and J. N. Gibbs. 1984. Colonization of elm bark by *Phomopsis oblonga*. Trans. Br. Mycol. Soc. 82:348−352. doi:10.1016/s0007-1536(84)80083-2

Integrated Disease Management to Reduce Disease Incidence in Exported Guava Fruits during Summer and Autumn

Shuen-Huang Tsai[1,*]

Abstract

Tsai, S. H. 2025. Integrated disease management to reduce disease incidence in exported guava fruits during summer and autumn. J. Taiwan Agric. Res. 74(3):331–343.

To reduce postharvest diseases of guava during storage and transportation, preharvest disease management is crucial for guava fruit exportation. A survey of diseases affecting guava (cv. Pearl) fruits in the field during the summer and autumn seasons revealed that black spot disease (caused by *Phyllosticta* spp.) is the primary cause of fruit rots, followed by anthracnose, scab, *Phytophthora* blight, and other fruit rots. Regarding fungal communities on asymptomatic leaves, fallen leaves, and branches in the field, *Phomopsis* spp. had the highest isolation rate, could be up to 100%. Additionally, common fruit pathogens such as *Phyllosticta* spp. were also isolated. Notably, 84.6% of the *Phyllosticta* isolates were pathogenic, indicating the potential for latent infections. Trials involving guava fungicides approved by the Animal and Plant Health Inspection Agency and commercially available microbial fungicides were evaluated to develop an integrated disease management strategy tailored specifically for summer and autumn fruit diseases in orchards supplying export markets. The strategy begins with orchard sanitation, including removal of fallen leaves and pruned branches, combined with the application of environmentally friendly materials such as lime sulfur or Bordeaux mixture sprayed on the soil surface. For chemical management in the field, it is recommended to use fungicides approved by both Taiwan and Canada or the United States, such as 25% azoxystrobin. This should be mixed with microbial agents like *Bacillus amyloliquefaciens* (CL3) or *B. velezensis* (BF). These treatments should be applied at 7- to 10-day intervals and repeated at least three times. Subsequently, rotation of fungicides with different modes of action combined with microbial products can help reduce the risk of fungicide resistance and minimize the likelihood of exceeding residue limits.

Key words: Exported guava, Guava black spot, Azoxystrobin, *Bacillus* spp., Integrated disease management.

Received: April 24, 2025; Accepted: July 22, 2025.

[*] Corresponding author, e-mail: tsh@tari.gov.tw

[1] Assistant Research Fellow, Department of Plant Protection and Utilization, Fengshan Tropical Horticultural Experiment Branch, Taiwan Agricultural Research Institute, Kaohsiung, Taiwan, ROC.

台灣農業研究 (J. Taiwan Agric. Res.) 74(3):345–357 (2025)
https://doi.org/10.6156/JTAR.202509_74(3).0011

研究報告

洛神葵疫病病原 *Phytophthora nicotianae* 的鑑定及抗病品種之篩選

蔡惠玲[1] 詹月梅[1] 許玴瑋[2] 黃晋興[3] 蔡志濃[4] 安寶貞[5] 陳敬文[6] 林筑蘋[7,*]

摘要

蔡惠玲、詹月梅、許玴瑋、黃晋興、蔡志濃、安寶貞、陳敬文、林筑蘋。2025。洛神葵疫病病原 *Phytophthora nicotianae* 的鑑定及抗病品種之篩選。台灣農業研究 74(3):345–357。

洛神葵為臺灣之重要特用作物，本研究於中部田間調查發現，罹患疫病的洛神葵在雨季後經常出現葉片失水下垂、莖基部呈現褐化，以及根部褐變腐敗，罹病後期全株呈乾枯狀，並造成嚴重損失。經調查，本研究分離獲得的洛神葵疫病菌近年均為 A^1 配對型，但早年分離的菌株均為 A^2 配對型。以形態特徵輔以分子序列，並經柯霍氏法則完成病原性測定，確定洛神葵疫病是由 *Phytophthora nicotianae* Breda de Haan 1896 (= *P. parasitica*) 所造成，且菌株致病力差異與傷口有無都可能會影響病害嚴重度。利用中度致病力分離株作為品種抗感病篩選的供試菌株，針對 7 種常見之洛神葵品種進行接種。不同洛神葵品種對於疫病菌具有不同程度的抗感病性，其中「臺東 2 號」、「臺東 3 號」及「臺東 4 號」等 3 個洛神葵品種具有較高的抗性，「大紅」與「斑馬」等 2 品種感病性最高，而「臺東 1 號」則為中等感病。

關鍵詞：洛神葵疫病、病原性測試、品種抗感病性。

前言

洛神葵 (*Hibiscus sabdariffa* L.) 英文名：Roselle，又名山茄、玫瑰茄、羅濟葵、紅葵及洛濟葵，俗稱洛神花 (Chen 2012)。洛神葵包括至少 2 個用途不同的變種：*H. sabdariffa* var. *altissima* Westerr 豐富的韌皮纖維是全球重要之經濟纖維來源；*H. sabdariffa* var. *sabdariffa* 肉質的果顎能製果凍、果醬及蜜餞等加工食品或烘乾泡茶飲用，普受國人歡迎，為臺灣常見的栽培變種 (Ansari *et al*. 2013; Chen 2022)。

在臺灣，洛神葵主要產區包括東部的臺東縣與花蓮縣、中部的南投縣與雲林縣，其中高達 7 成以上集中在臺東縣，為最大的栽培中心 (Chen 2022)。已知的洛神葵病害種類包括灰黴病 (病原 *Botrytis cinerea*)、角斑病 (病原 *Cercospora malayensis*)、炭疽病 (病原 *Colletotrichum gloeosporioides* s.l.)、菌核病 (病原 *Sclerotinia sclerotiorum*)、青枯病 (病原 *Ralstonia solanacearum*) (Tzean *et al*. 2019) 以及由 *Fusarium solani* 引起的萎凋病 (Wang *et al*. 2021) 等。此外 Yu (1975) 報告洛神葵立枯病 (roselle blight) 由疫病菌 *Phytophthora* sp. 引起，而現今則稱該病害為『洛神葵疫病』。

洛神葵疫病在中美洲、非洲及亞洲等地造成莫大的損失，曾造成田間 10–25%，甚至 40% 以上的產量損失；透過人工接種，亦能夠

投稿日期：2025 年 5 月 15 日；接受日期：2025 年 7 月 22 日。
* 通訊作者：cplin@tari.gov.tw
1 農業部農業試驗所植物病理組計畫助理。臺灣 臺中市。
2 國立屏東科技大學植物醫學系大學生。臺灣 屏東縣。
3 農業部農業試驗所植物病理組研究員。臺灣 臺中市。
4 農業部農業試驗所植物病理組研究員兼組長。臺灣 臺中市。
5 農業部農業試驗所植物病理組前研究員兼組長。臺灣 臺中市。
6 農業部臺東區農業改良場作物改良科助理研究員。臺灣 臺東縣。
7 農業部農業試驗所植物病理組助理研究員。臺灣 臺中市。

導致 100% 的接種植株死亡 (Rojas-Rojas *et al*. 2017; Swathi *et al*. 2025)。在墨西哥，洛神葵疫病被稱為 “black leg”，罹病植株會出現莖基部壞死，壞死之病徵有時會向上擴展到地上部分與部分枝條，進而造成地上部萎凋、黃化及葉片軟化，被視為種植洛神葵最重要的病害之一 (Ortega-Acosta *et al*. 2015)；在印度或其他地區，洛神葵疫病又稱為 “foot and stem rot”，或是 “root rots”，病徵包括根與莖基部黑褐化、地上部萎凋，最終導致植株死亡，嚴重影響產量 (Ansari *et al*. 2013; Swathi *et al*. 2025)。

在臺灣，最早的洛神葵疫病紀錄於 1974 年的臺中，病徵與前述墨西哥與印度案例相似，植株自幼株即會受害，到生育盛期亦易罹病，可導致全園嚴重受害 (Yu 1975)；之後，2018 年在臺東亦出現萎凋病徵之洛神葵根部可分離到疫病菌的記錄，初步鑑定為 *P. nicotianae* (Wang *et al*. 2019, 2021)。然而，關於臺灣洛神葵疫病病原相關的科學證據實並不完整，例如對於病原菌的特徵描述尚不完全，且仍有待完成柯霍式法則 (Huang *et al*. 2023)。

本研究自 1976–2023 年於田間採集出現疫病之洛神葵，調查地區涵蓋包括嘉義、臺南、雲林、南投及臺中等中部地區，並且完整記錄病原資料與完成柯霍式法則確定病原性。除透過形態與分子特徵鑑定所得之分離菌株外，同時測定病原之生理與病原性，例如菌株配對型、菌絲最適生長溫度，以及菌株致病力 (virulence) 差異與傷口有無對感染疫病的影響，並且初步篩選市面常見之洛神葵品種對疫病菌的抗感病性。本研究希冀透過建立洛神葵疫病基本資料，進而提供初步可能之防治方向。

材料與方法

病原菌之分離與保存

先將洛神葵罹病組織，包括根、莖及枝條等病組織洗淨、以紙巾瀝乾水分，接下來組織分離的方式如 Ann *et al*. (2018) 所述。分離出之疫病菌經單菌絲分離後，再次移殖於 5% V8 瓊脂 (5% V8 juice agar, 5% VA) 上，在 24℃ 下無光照培養 3–5 d，取前端菌絲塊 (10 mm × 5 mm × 5 mm) 保存於 20–24℃ 下含礦物油 (Mineral oil, Sigma Aldrich, Burlington, MA, USA) 之試管中備用。

供試菌株

自不同地區之罹病洛神葵上分離之 50 株分離菌株，詳細之供試菌株資料列於表 1。其他使用菌株包括美國加州大學 Dr. G. A. Zent-

表 1.　本研究自出現萎凋病徵之洛神葵所分離之 *Phytophthora nicotianae* 菌株清單。

Table 1.　List of the *Phytophthora nicotianae* obtained from roselle plants exhibiting wilt symptoms in this study.

Diseased roselle tissue	Year	Location	Species	Isolate no.	Mating type
Stem base	1976	East, Chiayi City	*P. nicotianae*	PNhi1-1	A^2
Stem base	1976	East, Chiayi City	*P. nicotianae*	PNhi1-2	A^2
Stem base	1976	East, Chiayi City	*P. nicotianae*	PNhi1-3	A^2
Stem base	1976	East, Chiayi City	*P. nicotianae*	PNhi1-4	A^2
Stem base	1976	East, Chiayi City	*P. nicotianae*	PNhi1-5	A^2
Seedling	1977	East, Chiayi City	*P. nicotiana*	PNhi2-1	A^2
Seedling	1977	East, Chiayi City	*P. nicotiana*	PNhi2-2	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-1	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-2	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-3	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-4	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-5	A^2

表 1.　本研究自出現萎凋病徵之洛神葵所分離之 *Phytophthora nicotianae* 菌株清單。(續)

Table 1.　List of the *Phytophthora nicotianae* obtained from roselle plants exhibiting wilt symptoms in this study. (continued)

Diseased roselle tissue	Year	Location	Species	Isolate no.	Mating type
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-6	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-7	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-8	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-9	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-10	A^2
Stem base	1978	Madou, Tainan City	*P. nicotiana*	PNhi3-11	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-6	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-7	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-8	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-9	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-10	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-11	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-12	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-13	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-14	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-15	A^2
Stem base	1980	East, Chiayi City	*P. nicotianae*	PNhi1-16	A^2
Stem base	1981	East, Chiayi City	*P. nicotianae*	PNhi1-17	A^2
Stem base	1981	East, Chiayi City	*P. nicotianae*	PNhi1-18	A^2
Stem base	1981	East, Chiayi City	*P. nicotianae*	PNhi1-19	A^2
Stem base	2001	Wufeng, Taichung City	*P. nicotianae*	p201210	A^1
Stem base	2001	Wufeng, Taichung City	*P. nicotianae*	p201211	A^1
Stem base	2001	Wufeng, Taichung City	*P. nicotianae*	p201212	A^1
Stem base	2001	Wufeng, Taichung City	*P. nicotianae*	p201213	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210386	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210387	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210388	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210389	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210394	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210395	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210396	A^1
Stem base	2010	Gukeng, Yunlin County	*P. nicotianae*	p210397	A^1
Stem base and root	2015	Zhongliao, Nantou County	*P. nicotianae*	p215097	A^1
Stem base and root	2015	Zhongliao, Nantou County	*P. nicotianae*	p215098	A^1
Stem base	2023	Linnei, Yunlin County	*P. nicotianae*	p223017	A^1
Stem base	2023	Linnei, Yunlin County	*P. nicotianae*	p223018	A^1
Stem base	2023	Linnei, Yunlin County	*P. nicotianae*	p223019	A^1
Stem base	2023	Linnei, Yunlin County	*P. nicotianae*	p223020	A^1

myer 早年贈送的標準菌株 *P. nicotianae* A^1 菌株 (p991) 與 A^2 菌株 (p731)，用於菌株配對型之檢測。

菌落形態觀察

將供試菌株 p210394 與 p223020 (表 1) 於室溫 (25℃) 下培養於 5% 離心 V8 之瓊脂 (5% centrifuged V8 agar; 5% CVA) 與馬鈴薯葡萄糖瓊脂 (potato dextrose agar; PDA, BD Difco™, Franklin Lakes, NJ, USA) 的玻璃培養皿 (直徑 9 cm；Pyrex, Corning Inc., Corning, NY, USA) 中，培養天數分別為 5–7 d 與 20–25 d。

疫病菌之培養與產孢

菌絲生長與溫度之關係

配製 5% CVA 平板，每培養皿 (直徑 9 cm) 中含有 20 mL 培養基。供試菌株先在 5% VA 室溫培養 3–5 d，以 0.6 cm 的打孔器在菌落先端切成菌絲塊，移入含 5% CVA 培養皿的一端 (約距邊緣 1 cm)。溫度分成 4、8、12、16、20、24、28、32 及 36℃ 等 9 處理，自第 2 日開始記錄菌絲的直線生長距離，至菌絲長滿培養皿或生長至第 7 日為止，統計各溫度自第 2 日起至最後一日之平均每日生長速度，確認菌絲之最適生長溫度。每處理 4 皿，試驗重複 3 次。

孢囊 (sporangia) 產生與游走子 (zoospores) 釋放

依 Hwang *et al*. (1975) 的方法，使供試菌株同步產生大量孢囊。孢囊長出後，在顯微鏡 (Olympus BX-50 Upright) 下觀察與測量其大小，並以 Canon 50D 相機拍攝形態，每菌株至少量測 30 個孢囊的大小。菌株同步形成大量孢囊後，每皿加入 20 mL 無菌水，於 16℃ 下靜置 30 min，再放回室溫 30 min (Hwang *et al*. 1975)。待游走子大量釋放後，倒入無菌燒杯，調整為 10^5 zoospores mL^{-1} 懸浮液，並將燒杯置入含冰塊的杯子內，作為接種備用。

厚膜孢子 (chlamydospores) 產生

取 5% VA 上培養 3–5 d 之供試菌株，將其先端菌絲切成 5 mm × 5 mm × 3 mm 小塊，移植於含有 20 mL 5% VA 的玻璃培養皿中，每皿放 5–7 個菌絲塊，24℃ 下無光照培養 4–7 d 後測量厚膜孢子大小，每菌株至少量測 30 個厚膜孢子。

配對型 (mating type) 與有性世代構造

依 Ann & Ko (1998) 的方法，將供試菌株分別與標準菌株 (*P. nicotianae*) p991 (A^1) 與 p731 (A^2) 於 24℃ 無光照對峙培養 7–14 d，於顯微鏡下鏡檢有無卵孢子 (oospores) 產生，以判斷測定供試菌株的配對型。可與 A^1 配對產生卵孢子者為 A^2 型；可與 A^2 配對形成卵孢子者為 A^1 型；如果自行形成卵孢子即為同絲型。異絲產孢者再進行薄膜配對 (Ko 1978)，約 20 d 後拍照並測量藏卵器、卵孢子及藏精器的構造與大小。每菌株至少量測 30 個不同的有性世代構造。

核醣體內轉錄區間 ITS 與 *cox I* 部分序列定序

萃取供試菌株之 gDNA，委託源資國際生物股份有限公司 (Tri-I Biotech Inc., New Taipei, Taiwan) 以特定引子對進行聚合酶鏈鎖反應 (polymerase chain reaction; PCR) 與定序。增幅的序列包括核醣體內轉錄區間 (internal transcribed spacer, ITS；引子對 ITS4/ITS5) 序列 (White *et al*. 1990)，與cytochrome c oxidase subunit I (*cox I*；引子對 COXF4N/COXR4N) 區間的部分基因序列 (Kroon *et al*. 2004)，所使用之引子對與 PCR 增幅條件如其文獻所列。

利用 Vector NTI 軟體 (Vector NTI software v.10.0, InforMax Inc., Bethesda, MD, USA) 工具將上述獲得的 ITS 與部分 *cox I* 基因序列分別經由接合 (concatenation) 與修剪 (trimming) 後獲得完整序列。定序後的序列上傳到 National Center for Biotechnology Information (NCBI, http://www.ncbi.nlm.nih.gov) 網站，利用BLAST (Basic Local Alignment Search Tool) 功能從 GenBank 資料庫中搜尋相似度最高的疫病菌種類與菌株序列代號，並將本研究獲得之 p210394 與 p223020 菌株的 ITS 與 *cox I* 序列登錄在 GenBank 資料庫中 (https://www.ncbi.nlm.nih.gov/genbank/)。

病原性測定

供試菌株包括 p210394 與 p223020。供試植物品種包括「臺東 1 號」、南投材明華先生提供之「大紅」與「斑馬」之洛神葵植株，供試時株齡約為 12 wk。種植方式為先將供試植株種子播種於培養土，發芽約 5 d 後移植於 6 吋盆中 (珍珠石：蛭石：培養土 = 1 : 1 : 3)，於 24–30℃ 溫室中生長，直至下述各實驗合適之株齡後供試。供試植物之莖基部先以酒精 (70% alcohol) 消毒，利用解剖刀 (feather double edge) 輕輕刮去靠近栽培土之莖基部表面組織以製造傷痍，傷痍處用消毒棉纏繞，將 10 mL 供試菌株之游走子懸浮液澆灌於消毒棉纏繞之傷痍上，再以鋁箔紙包覆並固定傷痍處。接種後全株罩塑膠袋 (60 cm × 30 cm) 並將袋口密封，培養於走入式定溫箱內 (24℃，RH 75–85%，12/12 h 光週期)，2 d 後移除塑膠袋。每2 d調查植株發病情形，至2 wk為止。每處理接種 5 株，試驗重複 2 次。對照處理接種無菌水。

不同品種 (品系) 之抗感病性測定

供試菌株為 p223020。供測的洛神葵株齡為 2 wk 大，品種包括「臺東 1 號」、「臺東 2 號」、「臺東 3 號」、「臺東 4 號」及「臺東 6 號」共 5 個品種 (Chen 2012; Chiang & Chen 2015; Chen 2022)，以及南投材明華先生提供之「大紅」與「斑馬」品種，種植與接種方法同上。每 2 d 調查植株發病情形，至 2 wk 為止。罹病級數 (disease index) 分為 0–4 共 5 級 (0：無病徵；1：莖基部褐化；2：莖基部縊縮；3：地上部萎凋；4：植株萎凋死亡)。每品種每次接種 5 株幼苗，試驗重複 3 次，共 15 株。每次的發病結果趨勢相似。將每品種的 15 株發病級數，利用 JASP 0.19.3.0 版統計分析軟體 (Love *et al*. 2019)，先進行無母數單因子變異數分析之 Kruskal-Wallis 檢定，確認 $P < 0.01$ 後，再以 Dunn's test 進行事後檢定，比較處理間平均值之差異，$P < 0.01$ 認定為具顯著性。最後以 Microsoft Office 365 中的 Excel 軟體繪製盒鬚圖 (box-and-whisker plot)。

結果

洛神葵疫病的病徵與危害狀況

自 1976 年起，嘉義、臺南、雲林、南投及臺中等多處果園之洛神葵出現地上部萎凋，葉片下垂、脫落 (圖 1A)，莖基部褐化縊縮等病徵；濕度高時罹病處常出現白色棉狀菌絲 (圖 1B)，且枝條亦可能受到感染而褐化縊縮 (圖 1C)。感染處之樹皮 (圖 1D) 或維管束與髓部亦會褐化 (圖 1E)。

疫病菌形態與生長特性

於 1976–2023 年間，自罹染疫病的洛神葵莖基與根部分離病原菌，共計 50 株 (表 1)，其菌落與孢囊特徵均類似，但配對型有所差異。1976–1981 年在嘉義與臺南分離得的 32 個分離株配對型為 A^2，此後 2001–2023 年在臺中、雲林及南投等地分離得的 18 個分離株配對型為 A^1。本研究以 p210394 與 p223020 為供試代表菌株，敘述其型態如下 (圖 2、表 2)。

在 5% CVA 上，p210394 與 p223020 之菌絲皆可在 4–36℃ 生長，最適生長溫度分別為 28℃ 與 32℃ (表 2)，兩者有些微差距。室溫下，在 5% CVA 平板培養基上 (圖 2A)，菌落邊緣出現些微的嵌紋狀花紋，氣生菌絲稀少 (圖 2A)；在 PDA 平板培養基上 (圖 2B)，菌落為瓣化 (petaloid) 到玫瑰花結狀 (rossette)，氣生菌絲較在 5% CVA 上多。孢囊以單假軸方式著生 (simple sympodial branching) (圖 2C)，每一孢囊梗可著生 5–7 個孢囊。孢囊為卵圓形 (ovoid) 或倒梨形 (obpyriform)，兩側對稱或稍不對稱，孢囊不具脫落性，具有明顯的乳突 (prominent papillae)，長 × 寬 46.2-(53.0)-61.5 × 38.4-(43.6)-49.2 [min.-(avg.)-max.] μm，長寬比為 1.1-(1.2)-1.4。藏卵器表面平滑，藏精器單生單室底著 (圖 2C)，藏卵器大小為 22.5-(26.2)-32.5 μm；卵孢子大小為 18.8-(22.5)-27.5 μm；藏精器大小為 10.0-(12.0)-12.5 × 7.5-(12.0)-17.5 μm。可產生厚膜孢子 (圖 2D)，大小為 25.0-(34.1)-45.0 μm。根據形態特徵，符合 *Phytophthora nicotianae* Breda de Haan 之描述 (Waterhouse 1963, 1970; Ho *et al*. 1995)。

圖 1.　洛神葵疫病病徵。(A) 受感染的洛神葵植株出現枯萎症狀；(B) 受感染的莖基部褐變，並出現白色菌絲；(C) 枝條被感染導致變色或壞死；(D) 受感染的樹皮橫切面上褐化；(E) 維管束與髓部褐化。

Fig. 1. Roselle blight caused by *Phytophthora nicotianae*. (A) Wilted symptoms of the infected roselle plant; (B) The infected stem exhibits constriction and browning, with white mycelium; (C) Upper stem infection showing discoloration or necrosis; (D) Browning of bark; and (E) Vascular and woody tissues in a cross-section of the infected branches.

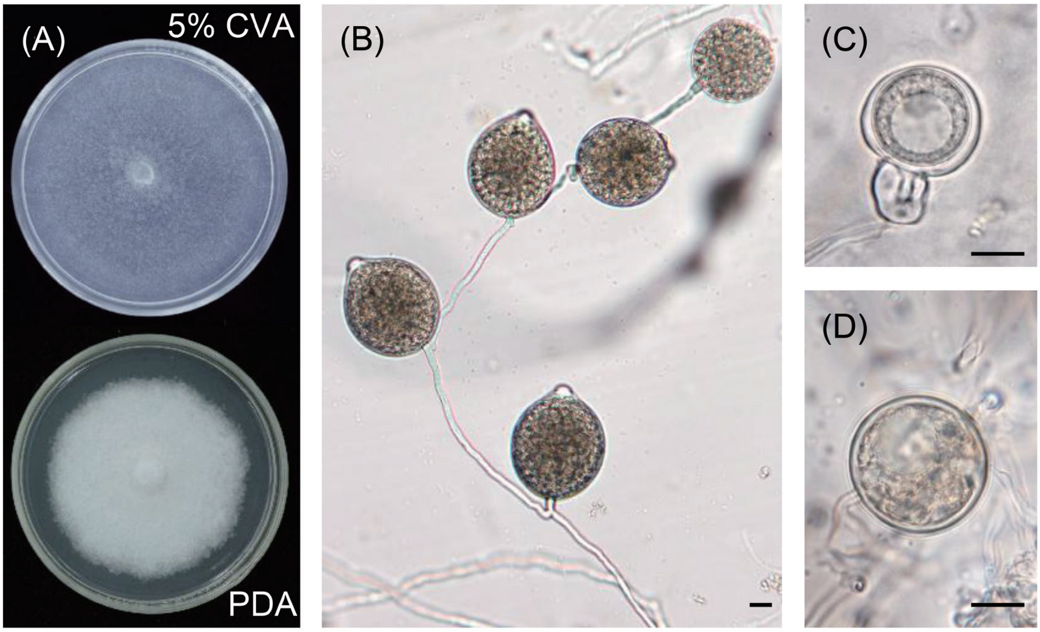

圖 2. *Phytophthora nicotianae* 代表菌株 p223020 之特徵。(A) 病原菌在培養基 (5% centrifuged V8 agar; CVA 與 potato dextrose agar; PDA) 上於 25℃下生長 7 d 的特徵；(B) 孢囊；(C) 穿雄生之藏卵器、卵孢子及下方的藏精器；(D) 厚膜孢子。

Fig. 2. Morphological features of the representative *Phytophthora nicotianae* isolate, p223020. (A) Colony morphologies on 5% centrifuged V8 agar (CVA) and potato dextrose agar (PDA) plates after 7 d growth at 25℃, with top view; (B) Sporangia with prominent papillae and displaying simple sympodial branching; (C) Oogonia and oospore with amphigynous antheridia; and (D) Abundant chlamydospores observed. Bar = 10 μm.

ITS 區間與 *cox I* 部分序列分析比對

藉由 NCBI 網站 GenBank 資料庫比對供試菌株 p210394 與 p223020 之 ITS 區間與 *cox I* 基因部分片段 (表 2)，供試菌株之 ITS 區域序列皆與 *P. nicotianae* 2013CAP2002 (accession no. KJ549640) 序列相似度為 100%；供試菌株之 *cox I* 的基因序列亦皆與 *P. nicotianae* P1976 (accession no. DAZ88467) 序列相似度為 100%。本試驗結果顯示 *P. nicotianae* 基因序列的比對結果與形態鑑定結果具一致性。

疫病菌病原性測試

在株齡 12 wk 的洛神葵「大紅」植株莖基部，分別澆灌接種 *P. nicotianae* p223020 與 p210394 菌株的游走孢子懸浮液。人工傷痍處理約 2 wk 後，接種點開始出現與田間相似的病徵 (圖 3)，包括地上部萎凋、部分葉片下垂及落葉；莖基部自傷口處往上下擴散出現褐化縊縮與維管束褐化，部分供試植株接種處出現白色菌絲；根系壞疽、細根減少。改以同為株齡 12 wk 的洛神葵品種「斑馬」與「臺東 1 號」為供試植株。接種 2 wk 後，植株無發病或僅部分出現輕微的萎凋，莖基部出現不明顯或輕微的壞疽 (圖 3)。

改以 p210394 作為供試菌株亦可得相似的結果，但「大紅」與「斑馬」供試植株在接種後的病徵更嚴重，包括地上部萎凋、莖基褐化壞疽縊縮、維管束褐化，及根部壞疽等病徵更明顯；而「臺東 1 號」較抗病，在接種後的病徵則維持輕微或不明顯 (圖 3)。

前述發病植株與發病部位，經組織分離後可再分離得相同病原，完成柯霍氏法則 (Koch's postulates)。無人工傷痍處理或接種無菌水的對照組則無明顯病徵，且組織分離亦無法分離獲得病原菌。

表 2.　洛神葵疫病病原 *Phytophthora nicotianae* 代表菌株之序列資訊、產孢構造大小及最適生長溫度。

Table 2.　Sequence information, sporulation structure size, and optimal growth temperature of the representative *Phytophthora nicotianae* isolates from roselle blight.

Isolate no.	Accession no.		Sporangia (μm)[z]			Oogonia (μm)	Oospores (μm)	Antheridia (μm)		Chlamydospores (μm)	Optimum temperature for mycelium growth (℃)
	ITS	*cox I*	Length	Width	L/W			Length	Width		
p210394	PV123966	PV133357	45.0-(51.5)-60.0	30.0-(37.5)-45.0	1.1-(1.4)-1.6	25.0-(29.8)-35.0	22.5-(27.0)-32.5	10.0-(11.8)-12.5	7.5-(13.0)-15.0	25.0-(33.8)-45.0	28
p223020	PV123967	PV133358	46.2-(53.0)-61.5	38.4-(43.6)-49.2	1.1-(1.2)-1.4	22.5-(26.2)-32.5	18.8-(22.5)-27.5	10.0-(12.0)-12.5	7.5-(12.0)-17.5	25.0-(34.1)-45.0	32

[z] $N \geq 30$; the size of the structures mention in the list were presented as "min.-(avg.)-max".

不同洛神葵品種品系對 *P. nicotianae* 之抗感病性測定

為瞭解不同品種之洛神葵對 *P. nicotianae* 的抗感病性，蒐集臺灣中、南及東部常見之 7 種洛神葵品種，並以株齡 2 wk 的植株供試，並以中度致病能力的分離株 p223020 作為品種抗感病篩選時的供試菌株。結果顯示 (圖 4)，供測品種中，「大紅」(DH)、「斑馬」(ZB) 及「臺東 6 號」(T6) 在接種 *P. nicotianae* 後的平均罹病級數介於 3.1–3.9 間，感病性顯著地高於其他供試品種，病徵多為植株萎凋死亡或地上部萎凋 (罹病級數個別為 4 與 3)，少數為輕微的莖基部縊縮 (罹病級數 2)；「臺東 2 號」(T2)、「臺東 3 號」(T3) 及「臺東 4 號」(T4) 之罹病級數平均罹病級數介於 2.3–2.4 間，感病性顯著地低於其他供試品種，病徵多為輕微的莖基部縊縮，部分為地上部萎凋，少數出現植株萎凋死亡，亦有少部分僅接種處出現褐化 (罹病級數 1)；「臺東 1 號」(T1) 平均罹病級數為 2.8，罹病嚴重度雖然未若「大紅」、「斑馬」及「臺東 6 號」高，但仍稍高於「臺東 2 號」、「臺東 3 號」及「臺東 4 號」，顯示「臺東 1 號」感病性為介於供試品種中間，病徵多為地上部萎凋與輕微的莖基部縊縮，部分植株萎凋死亡，少數僅接種處出現褐化。以上結果經 Kruskal-Wallis 檢定及 Dunn's 事後檢定分析後顯示，品種間差異達顯著水準 ($P < 0.01$)。

討論

臺灣自 1974 起，即有由 *Phytophthora* sp. 引起的洛神葵疫病害紀錄 (Yu 1975)，本研究進一步補充與完善相關資料。本研究於 1976–2023 年，在中部的縣市採集發病的洛神葵疫病植物組織，藉由組織分離皆取得形態一致的菌株。經過形態與序列鑑定，確定獲得的菌株為 *P. nicotianae* (表 1)。進一步透過完成科霍氏法則，證實臺灣的洛神葵疫病是由 *P. nicotianae* 引起。

洛神葵疫病與洛神葵萎凋病的病徵相似，可以區別之處在於洛神葵疫病在莖基部形成白色菌絲，而洛神葵萎凋病則是形成白至黃色菌

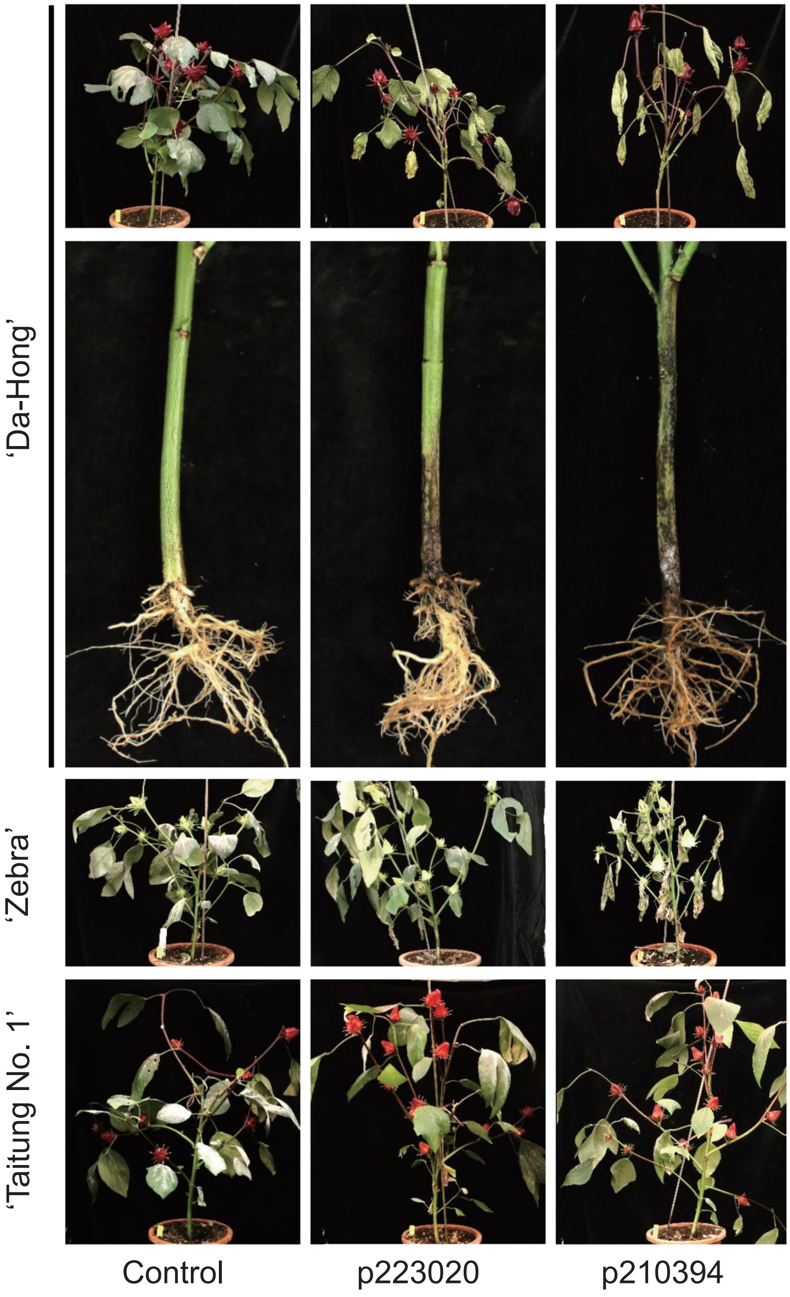

圖 3. 洛神葵疫病病原 *Phytophthora nicotianae* (分離株 p223020 與 p210394) 之病原測試。供試植物包括「大紅」、「斑馬」以及「臺東 1 號」。植株株齡 12 wk 個別澆灌以 10 mL 對應菌株的游走子懸浮液 (10^5 zoospores mL^{-1})。每處理每次接種 5 個植株，每個試驗重複 2 次。對照組以無菌水取代游走子懸浮液。接種 2 wk 後記錄病徵表現。

Fig. 3. Pathogenicity test of *Phytophthora nicotianae* (isolates p223020 and p210394) on roselle cultivars. The tested cultivars included 'Da-Hong', 'Zebra', and 'Taitung No. 1'. Twelve-week-old roselle plants were wounded at the basal stem and inoculated with 10 mL of zoospore suspension (10^5 zoospores mL^{-1}) of each tested isolates. Each treatment included 5 replicates, and the experiment was repeated twice. Control plants were inoculated with sterile water. Disease symptoms were recorded 2 wk post-inoculation.

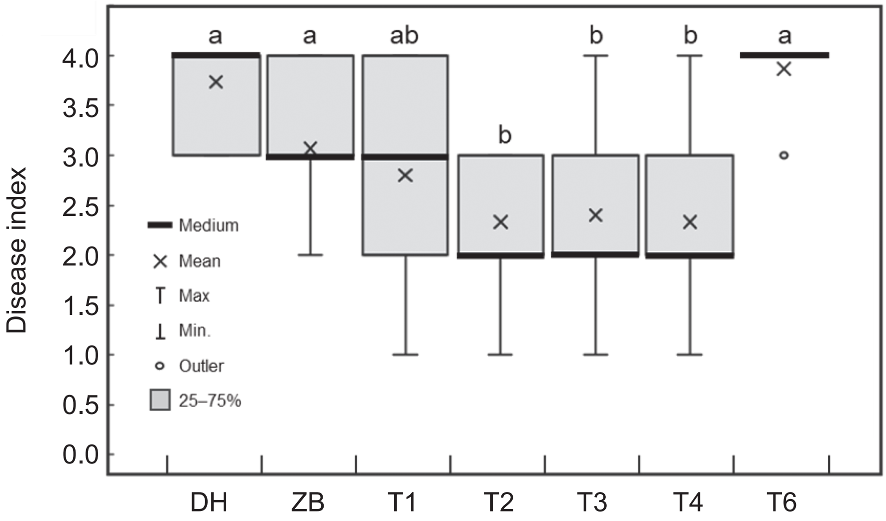

圖 4.　不同洛神葵品種接種 *Phytophthora nicotianae* (分離株 p223020) 2 wk 後之罹病嚴重程度。以株齡 2 wk 的植株進行試驗，每品種每次接種 5 個植株，每個試驗重複 3 次。每次的發病結果趨勢相似，將每品種的 15 株發病級數數據集合進行統計分析。罹病級數分為 0–4：0 = 無病徵；1 = 莖基部褐化；2 = 莖基部縊縮；3 = 地上部萎凋；4 = 植株萎凋死亡。柱狀圖上方之不同字母代表經 Kruskal-Wallis 檢定與 Dunn's 事後比較分析後，各品種在統計上具顯著差異 ($P < 0.01$)。DH、ZB、T1、T2、T3、T4 及 T6 分別代表「大紅」、「斑馬」、「臺東 1 號」、「臺東 2 號」、「臺東 3 號」、「臺東 4 號」及「臺東 6 號」等品種。

Fig. 4.　Disease severity of different roselle cultivars following inoculation with *Phytophthora nicotianae* (isolate p223020) 2 wk post-inoculation. The experiment was conducted on 2-week-old plants and repeated 3 times, with 5 replicates for each cultivar in each trial. As the disease severity trends were consistent across the three replicates, data from all 15 plants per cultivar were pooled for statistical analysis. Disease severity was evaluated using a 0 to 4 disease index: 0 = no symptoms; 1 = browning at the stem base; 2 = constriction at the stem base; 3 = wilting of aerial parts; and 4 = complete plant wilting and death. Different letters above the bars indicate statistically significant differences among cultivars based on the Kruskal-Wallis test followed by Dunn's test ($P < 0.01$). DH, ZB, T1, T2, T3, T4, and T6 represent the cultivars 'Da-Hong', 'Zebra', and 'Taitung No. 1, 2, 3, 4, and 6', respectively.

絲。然而，兩病害在發病前期的症狀極為相似，皆為地上部萎凋、莖基部褐化及維管束變色。在洛神葵萎凋的調查中，也常發現 *P. nicotianae* 與 *Fusarium solani* 同時存在 (Ortega-Acosta *et al*. 2015)。洛神葵主要產區集中在臺東縣 (Chen 2022)，Wang *et al*. (2021) 普查臺東縣內洛神葵出現地上部萎凋病徵的發病原因，近 5 成以上的組織可分離得病原菌 *F. solani*，而僅約 1 成左右的組織分離得 *P. nicotianae*。Yu (1975) 以及本研究 1976–2023 年的調查，採樣地點皆是在中部地區 (表 1)，於出現萎凋病徵的洛神葵上皆一致分離獲得 *P. nicotianae*。由此可知，中部與東部的洛神葵病害相可能略有差異，中部地區長期以來可能以 *P. nicotianae* 為主要病原，而東部則以 *F. solani* 為主要病原。兩地區對於出現萎凋的洛神葵植株可能要有不一樣的防治策略。

細究本研究之田間調查，可看到早期於嘉義與臺南等地分離的洛神葵疫病 *P. nicotianae* 分離株配對型皆為 A^2，而後期在臺中、雲林、以及南投等地分離得的菌株配對型則為 A^1。雖尚未能釐清配對型變化是因為菌株族群變遷的緣故，或是區域性差異，但可以知道在臺灣 *P. nicotianae* A^1 與 A^2 兩種配對型皆可引起洛神葵疫病。根據在發生地點的觀察，無論是哪種配對型皆可引起嚴重的洛神葵疫病，調查的田區洛神葵疫病發病比例高且受害嚴重。

本研究中，*P. nicotianae* 洛神葵分離株 p210394 與 p223020 呈現不同的致病毒力 (圖 3)。以接種 12 wk 的植株為例，分離株 p223020

感染「大紅」後可造成植株中度萎凋，而感染「臺東 1 號」與「斑馬」則僅造成輕微到不明顯的萎凋，顯示分離株 p223020 展現出中度的致病力；分離株 p210394 感染「大紅」與「斑馬」後皆造成植株嚴重萎凋，顯示分離株 p210394 展現出高度的致病力。除了分離株間致病力差異性可影響罹病度外，高致病分離株 p210394 感染「臺東 1 號」後，植株僅出現輕微到不明顯的萎凋，與中度致病分離株 p223020 的結果相似，顯示植株品種抗性對病害管理相當重要。本研究亦發現，唯經過傷痍處理的植株才會發病 (未顯示之資料)，若未經過傷痍處理者則不發病，顯示傷口亦是該病害發生的關鍵之一。因為分離株 p210394 具有極強的致毒能力，為避免因使用致毒力強的菌株而無法區分不同品種的洛神葵對 *P. nicotianae* 的抗感病性關係，因此後續選用致毒力中等的 p223020 菌株，作為品種抗感病篩選時的供試菌株。

洛神葵的品種多元，經過長期演變，衍生出性狀各異的地方品系，其命名與品系混雜，如本次蒐集包括中部地區常見的「大紅」與「斑馬」品種。另蒐集由臺東區農業改良場育成、已知性狀及遺傳背景的洛神葵品種，除「臺東 5 號」因故未加入測試外，其他「臺東 1 號」、「臺東 2 號」、「臺東 3 號」、「臺東 4 號」及「臺東 6 號」品系皆列入供測。以 *P. nicotianae* p223020 菌株接種不同品種的洛神葵結果顯示，「大紅」、「斑馬」及「臺東 6 號」具較高的感病性，植株出現萎凋、倒伏，甚至死亡；「臺東 2 號」、「臺東 3 號」及「臺東 4 號」則具低感病性，植株僅出現莖基縊縮或程度不一的萎凋；而「臺東 1 號」介於兩者之間 (圖 4)。另外，比較圖 3 與圖 4 的罹病狀況可知，當供測品種「大紅」、「臺東 1 號」以及「斑馬」株齡由 2 wk 增加為 12 wk 時，雖然同樣接種 *P. nicotianae* p223020 菌株，但供試植株的罹病度都會降低。其中，「大紅」僅出現中度萎凋，「斑馬」與「臺東 1 號」嚴重度降為輕微或是不明顯 (圖 3)，推測可能具成株抗性的趨勢，有待未來進一步測試。

雖然決定洛神葵各品種抗疫病的效果，仍有待進一步於田間試驗評估 (Rojas-Rojas *et al*. 2017; Swathi *et al*. 2025)，但由本研究可知，菌株致病力、有無傷口以及洛神葵品種等因子，都會影響植株對於 *P. nicotianae* 的感病性。

本研究以科學的證據確認 *P. nicotianae* 為造成洛神葵疫病的主要病原，其在田間包含 2 種可進行有性世代的配對型。透過病原性以及品種感病性測試，建議田間管理時應特別注意品種的選擇，同時注意避免害蟲咬傷、颱風等災害，或其他原因造成的傷口，以降低洛神葵疫病發生的風險。

誌謝

感謝本組王如玉技工協助蒐集洛神葵品種。本研究經費承蒙農業部經費支持，計畫編號 114 農科-5.3.1-農 01 (4)。

引用文獻

Ann, P. J. and W. H. Ko. 1988. Hormonal heterothallism in *Phytophthora parasitica*: A novel mode of sexual re-production? J. Gen. Microbiol. 134:2985–2992. doi:10.1099/00221287-134-11-2985

Ann, P. J., C. P. Lin, J. N. Tsai, and H. L. Tsai. 2018. Newly recorded diseases of ornamental plants caused by *Phytophthora palmivora* in Taiwan. J. Plant Med. 60:21–28. (in Chinese with English abstract) doi:10.6716/JPM.201812_60(3_4).0003

Ansari, M., T. Eslaminejad, Z. Sarhadynejad, and T. Eslaminejad. 2013. An overview of the roselle plant with particular reference to its cultivation, diseases and usages. Eur. J. Med. Plants 3:135–145. doi:10.9734/EJMP/2013/1889

Chen, J. F. 2012. Breeding of the new roselle varieties Taitung 1, 2, 3. Res. Bull. Taitung Dist. Agric. Res. Ext. Stn. 22:53–65. (in Chinese with English abstract) doi:10.6959/RBTDAIS.201209.0053

Chen, J. W. 2022. Breeding of roselle ‘Taitung No.6 -Black Crystal’. Res. Bull. Taitung Dist. Agric. Res. Ext. Stn. 32:1–14. (in Chinese with English abstract)

Chiang, P. S. and J. F. Chen. 2015. Breeding of the roselle variety ‘Taitung 4 -Red Zebra’. Res. Bull. Taitung Dist. Agric. Res. Ext. Stn. 25:1–12. (in Chinese with English abstract)

Ho, H. H., P. J. Ann, and H. S. Chang. 1995. The Genus

Phytophthora in Taiwan. Ins. Bot., Acad. Sin. Mo. Ser. 15. Taipei, Taiwan. 86 pp.

Huang, J. H., C. P. Lin, C. W. Huang, J. N. Tsai, and P. J. Ann. 2023. *Phytophthora* species in Taiwan and their host range: A review. J. Taiwan Agric. Res. 72:289–316. (in Chinese with English abstract) doi:10.6156/JTAR.202312_72(4).0002

Hwang, S. C., W. H. Ko, and M. Aragaki. 1975. A simplified method for sporangial production by *Phytophthora cinnamomi*. Mycologia. 67:1233–1234. doi:10.1080/00275514.1975.12019873

Ko, W. H. 1978. Heterothallic *Phytophthora*: Evidence for hormonal regulation of sexual reproduction. J. Gen. Microbiol. 107:15–18. doi:10.1099/00221287-107-1-15

Kroon, L. P. N. M., E. C. P. Verstappen, L. F. F. Kox, W. G. Flier, and P. J. M. Bonants. 2004. A rapid diagnostic test to distinguish between American and European populations of Phytophthora ramorum. Phytopathology 94:613–620. doi:10.1094/PHYTO.2004.94.6.613

Love, J., R. Selker, M. Marsman, T. Jamil, D. Dropmann, J. Verhagen, ... E. J. Wagenmakers. 2019. JASP: Graphical statistical software for common statistical designs. J. Stat. Softw. 88(2):1–17. doi:10.18637/jss.v088.i02

Ortega-Acosta, S. Á., J. Hernández-Morales, J. S. Sandoval-Islas, V. Ayala-Escobar, L. Soto-Rojas, and A. Alejo-Jaimes. 2015. Distribution and frequency of organisms associated to disease “black leg” of roselle (*Hibiscus sabdariffa* L.) in Guerrero, México. Rev. Mex. Fitopatol. 33:173–194. (in Spanish with English abstract)

Rojas-Rojas, R., V. H. Aguilar-Rincón, C. D. L. G. de Alba, C. Velasco-Cruz, E. Valadez-Moctezuma, J. Hernández-Morales, and F. de Jesús Caro-Velarde. 2017. Response of native roselle varieties (*Hibiscus sabdarifa* L.) to infection by *Phytophthora parasitica* Dastur in Mexico. Acad. J. Biotechnol. 5:172–179. doi:10.15413/ajb.2017.0306

Swathi, B., Y. Rajasekhar, G. Sreenivas, R. M. Srinivas, R. Y. Sandhya, and B. G. Chitti. 2025. Evaluation of roselle (*Hibiscus sabdariffa* L.) varietal responses to foot and stem rot caused by *Phytophthora parasitica* var. *sabdariffae*. Arch. Curr. Res. Intl. 25:288–294. doi:10.9734/acri/2025/v25i21086

Tzean, S. S., K. C. Tzeng, C. A. Chang, T. T. Tsay, and H. F. Yen (eds.) 2019. List of Plant Diseases in Taiwan. 5th ed. Taiwan Phytopathology Society. Taichung, Taiwan. 329 pp. (in Chinese)

Wang, C. W., J. Y. Fong, Y. H. Hou, and Y. L. Chen. 2019. Identification of roselle wilt disease and development of organic control materials. J. Plant Med. 61:78. (in Chinese)

Wang, C. W., Y. H. Yu, C. Y. Wu, R. Y. Feng, K. Tandon, Y. L. Chen, and S. L. Tang. 2021. Detection of pathogenic and beneficial microbes for roselle wilt disease. Front. Microbiol. 12:756100. doi:10.3389/fmicb.2021.756100

Waterhouse, G. M. 1963. Key to the Species of *Phytophthora* de Bary. Mycological Papers. Volume 92. Commonwealth Mycological Institute, Kew Surrey, England. 22 pp.

Waterhouse, G. M. 1970. The Genus *Phytophthora* de Bary: Diagnoses (or descriptions) and Figures from the Original Papers. Mycological Papers. Volume 122. Commonwealth Mycological Institute, Kew Surrey, England. 59 pp.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p.315–322. *in*: PCR Protocols: A Guide to Methods and Applications. (Innis, M. A., D. H. Gelfand, J. J. Snirsky, and T. J. White, eds.) Academic Press. San Diego, CA. 482 pp. doi:10.1016/B978-0-12-372180-8.50042-1

Yu, K. S. 1975. First report of roselle blight. J. Plant Pathol, Natl. Chung Hsing Univ. 6:112–144. (in Chinese)

Identification of *Phytophthora nicotianae*, the Causal Agent of Roselle Blight and Screening for Resistant Cultivars

Hui-Ling Tsai[1], Yue-Mei Zhan[1], Yi-Wei Hsu[2], Jin-Hsing Huang[3], Jyh-Nong Tsai[4], Pao-Jan Ann[5], Jing-Wen Chen[6], and Chu-Ping Lin[7,*]

Abstract

Tsai, H. L., Y. M. Zhan, Y. W. Hsu, J. H. Huang, J. N. Tsai, P. J. Ann, J. W. Chen, and C. P. Lin. 2025. Identification of *Phytophthora nicotianae*, the causal agent of roselle blight and screening for resistant cultivars. J. Taiwan. Agric. Res. 74(3):345–357.

Roselle (*Hylocereus* spp.) is an important specialty crop in Taiwan. A field survey conducted in central Taiwan revealed that during the rainy season, roselle plants frequently developed blight symptoms including wilting, browning at the base of the stems, and root rot with brown discoloration. At an advanced stage, affected plants were desiccated and died resulting in severe yield loss. Most isolates of the roselle blight pathogen obtained in recent years were identified as A^1 mating type, while those isolated earlier were A^2 mating type. Pathogen identification was performed using morphological traits and molecular sequencing, and with the fulfillment of Koch's postulates *Phytophthora nicotianae* (= *P. parasitica*) was confirmed as the causal agent. Pathogenicity varied among isolates, and disease severity were influenced by factors such as the presence of wounds. Resistance screening using moderately virulent isolates showed that roselle cultivars differ in their susceptibility to *P. nicotianae*. Among the 7 common cultivars tested, 'Taitung No. 2', 'Taitung No. 3', and 'Taitung No. 4' exhibited the highest level of resistance, while 'Da-Hong' and 'Zebra' were the most susceptible ones. 'Taitung No. 1' displayed a moderate level of susceptibility.

Key words: Roselle blight, Pathogenicity test, Variety resistance and susceptibility.

Received: May 15, 2025; Accepted: July 22, 2025.

* Corresponding author, e-mail: cplin@tari.gov.tw
[1] Project Assistants, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[2] Undergraduate Student, Department of Plant Medicine, National Pingtung University of Science and Technology, Pingtung County, Taiwan, ROC.
[3] Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[4] Research Fellow and Division Director, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[5] Former Research Fellow and Division Director, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.
[6] Assistant Research Fellow, Crop Improvement Section, Taitung District Agricultural Research and Extension Station, Taitung County, Taiwan, ROC.
[7] Assistant Research Fellow, Plant Pathology Division, Taiwan Agricultural Research Institute, Taichung City, Taiwan, ROC.

Editorial Board

Editor-in-Chief
Shih, Hsien-Tzung
Division of Applied Zoology, Taiwan Agricultural Research Institute

Deputy Editor-in-Chief
Syu, Chien-Hui
Division of Agricultural Chemistry, Taiwan Agricultural Research Institute
Wu, Dong-Hong
Department of Agronomy, National Chung Hsing University

Editors
Ann, Pao-Jen
Division of Plant Pathology, Taiwan Agricultural Research Institute
Chang, Ruey-Jang
Agricultural Chemicals Research Institute
Chang, Yung-Ho
Department of Food and Nutrition, Providence University
Chen, Chien-Chung
Division of Applied Zoology, Taiwan Agricultural Research Institute
Chen, Chiling
Division of Agricultural Chemistry, Taiwan Agricultural Research Institute
Chen, Iou-Zen
Department of Horticulture and Landscape Architecture, National Taiwan University
Chen, Suming
Department of Bio-Industrial Mechatronics Engineering, National Taiwan University
Chung, Wen-Hsin
Department of Plant Pathology, National Chung Hsing University
Do, Yi-Yin
Department of Horticulture and Landscape Architecture, National Taiwan University
Hou, Feng-Nan
Department of Entomology, National Chung Hsing University
Huang, Biing-Wen
Department of Applied Economics, National Chung Hsing University
Huang, Chao-Chia
Division of Crop Science, Taiwan Agricultural Research Institute
Huang, Jenn-Wen
Department of Plant Pathology, National Chung Hsing University
Huang, Yuh-Ming
Department of Soil and Environmental Sciences, National Chung Hsing University
Juang, Kai-Wei
Department of Agronomy, National Chung Hsing University
Ku, Hsin-Mei
Department of Agronomy, National Chung Hsing University
Kuo, Bau-Jeng
Department of Agronomy, National Chung Hsing University
Lin, Huey-Ling
Department of Horticulture, National Chung Hsing University
Lin, Yann-Rong
Department of Agronomy, National Taiwan University
Liu, Li-Yu
Department of Agronomy, National Taiwan University
Lu, Hsiu-Ying
Miaoli District Agricultural Research and Extension Station
Lu, Kuang-Hui
Department of Entomology, National Chung Hsing University
Tsay, Jyh-Rong
Taiwan Agricultural Research Institute
Tzou, Yu-Min
Department of Soil and Environmental Sciences, National Chung Hsing University
Wang, Chan-Sen
Department of Agronomy, National Chung Hsing University
Yang, Chwen-Ming
Division of Crop Science, Taiwan Agricultural Research Institute
Yang, Jeng-Tze
Department of Entomology, National Chung Hsing University
Yen, Gow-Chin
Department of Food Science and Biotechnology, National Chung Hsing University

English Editorial Board
Chang, Chung-Jan
Department of Plant Pathology, University of Georgia, USA
Chung, Kuang-Ren
Department of Plant Pathology, National Chung Hsing University
Wang, Yin-Tung
Department of Horticultural Sciences, Texas A&M University, USA
Yang, Chin-Cheng Scotty
Department of Entomology, Virginia Polytechnic Institute and State University, USA

Invited Editor
Chung, Chia-Lin
Department of Plant Pathology and Microbiology, National Taiwan University

Editorial Assistants
Wang, Li-Jung
Editorial Office of Journal of Taiwan Agricultural Research

JOURNAL OF TAIWAN AGRICULTURAL RESEARCH

Vol. 74 No. 3 September 30, 2025

Published by Taiwan Agricultural Research Institute, Ministry of Agriculture
Publisher Dennis Wang
Editor-in-Chief Hsien-Tzung Shih
Address No.189, Zhongzheng Rd., Wufeng Dist., Taichung City 413008, Taiwan, ROC
Website https://www.tari.gov.tw/jtar; E-mail: jtar@tari.gov.tw; Tel: +886-4-2331-7236
Submission https://www.ipress.tw/J0042
Complete instructions to authors are available on Journal of Taiwan Agricultural Research website: https://www.tari.gov.tw/jtar/

Press Ainosco Press
Address 18F, No.80, Sec.1, Chenggong Rd., Yonghe Dist., New Taipei City 234634, Taiwan, ROC
Tel +886-2-2926-6006
E-mail press@airiti.com

Price NT$ 175 (per copy)
Distributor 1. GB Books / 1F., No.209, Songjiang Rd., Taipei City 104472, Taiwan, ROC
GB Website: http://www.govbooks.com.tw; Tel: +886-2-2518-0207
2. Wunan Books / No.6, Zhongshan Rd., Taichung City 400002, Taiwan, ROC; Tel: +886-4-2226-0330